

## OPENING LECTURE

### OL

#### INTEGRATIVE PROPERTIES OF SPINAL INTERNEURONS

**Jankowska E.**

Department of Neuroscience and Physiology, Göteborg University, Göteborg, Sweden.

The lecture will focus on processing that occurs at the level of spinal interneurons in parallel with that traditionally attributed to supraspinal neurons. It will also focus on those properties of spinal interneurons that could be targeted while trying to use the existing interneuronal networks to the maximum during motor rehabilitation.

## PLENARY LECTURES

### L1

#### SYNAPSE REARRANGEMENTS IN LEARNING AND MEMORY

**Caroni P.**

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.

Learning is correlated with the assembly of new synapses, but the roles of synaptogenesis processes in memory are poorly understood. I will discuss recent evidence that learning-related synapse rearrangements have critical roles for the efficiency and precision of learning and memory in the adult. First, I will show how synapse disassembly and the establishment of new synapses are both critically important for augmented long-term learning and memory upon environmental enrichment. Enrichment enhanced the disassembly and assembly of dynamic subpopulations of synapses. Upon enrichment, stable assembly of new synapses depended on the presence of  $\beta$ -Adducin, disassembly involved  $\beta$ -Adducin phosphorylation through PKC, and both were required for augmented learning. In the absence of  $\beta$ -Adducin enrichment still led to an increase in spine structures, but the assembly of synapses at those spines and learning were compromised. Virus-mediated re-expression of  $\beta$ -Adducin in hippocampal granule cells of  $\beta$ -Adducin<sup>-/-</sup> mice rescued new synapse assembly and learning upon enrichment. Second, I will show how a learning-related doubling in the numbers of hippocampal mossy fiber synapses that promote feedforward inhibition is critically important for the precision of hippocampus-dependent memories. One-trial and incremental learning led to robust, circuit-specific, long-lasting and reversible increases in the numbers of filopodial synapses onto fast-spiking interneurons that trigger feedforward inhibition in both hippocam-

pus and cerebellum. For contextual fear conditioning and Morris water-maze learning, increased feedforward inhibition connectivity by hippocampal mossy fibers had a critical role for the precision of the memory and the learned behavior. In the absence of mossy fiber LTP in Rab3a<sup>-/-</sup> mice, c-Fos ensemble re-organization and feedforward inhibition growth were both absent in CA3 upon learning, and the memory was imprecise. By contrast, in the absence of  $\beta$ -Adducin c-Fos re-organization was normal, but feedforward inhibition growth was abolished. In parallel, c-Fos ensembles in CA3 were greatly enlarged, and the memory was imprecise. Feedforward inhibition growth and memory precision were both rescued by re-expression of  $\beta$ -Adducin specifically in hippocampal mossy fibers. Finally, I will discuss how the pronounced alterations in the numbers of defined synapses revealed by these studies provide structural readouts to investigate the specific contributions of individual systems to learning.

### L2

#### DEEP BRAIN STIMULATION OF THE POSTERIOR HYPOTHALAMIC NUCLEUS AS AN ALTERNATE THERAPEUTIC TARGET FOR PARKINSON'S DISEASE

**Bland B.H.**

Behavioral Neuroscience Research Group, Department of Psychology, The University of Calgary, Calgary, Canada.

The posterior hypothalamic nucleus is a major component of the ascending brainstem hippocampal synchronizing pathways. The sensorimotor integration model asserts that components of the neural circuitry in hippocampus and associated structures function in the capacity of providing voluntary motor systems with continually updated feedback on their performance relative to changing environmental (sensory) conditions. A crucial aspect of this performance is the intensity with which the motor programs are initiated and maintained. The components of the neural circuitry involved in sensorimotor integration are those underlying the production of oscillation and synchrony (theta) in the hippocampus and associated structures. The talk will present an overview of theta band oscillation and synchrony and how it led to our testing of posterior hypothalamic stimulation in two rodent models of Parkinson's disease.

### L3

#### A NANOSCALE VIEW INTO THE DYNAMIC OF AMPA RECEPTOR ORGANIZATION IN SYNAPSES

**Choquet D.**

Institut Interdisciplinaire de Neurosciences, Université de Bordeaux, Bordeaux, France.

Ionotropic AMPA glutamate receptors (AMPA) mediate fast excitatory synaptic transmission in the central nervous system.

Using a combination of high resolution single molecule imaging techniques and video-microscopy, we have previously established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion. Single molecule approaches give access to the full distribution of molecule behaviors and overcome the averaging intrinsic to bulk measurement methods. They allow access to complex processes where a given molecule can have heterogeneous properties over time. We will present some recent developments in single molecule imaging technologies and their application to track single molecules in live neurons. We have recently found a new function for this fast diffusion in controlling fast synaptic transmission. Upon consecutive synaptic stimulation at high frequency, synaptic transmission is depressed. This depression shapes the frequency dependent adaptation of individual synapses. AMPAR lateral diffusion allows fast exchange of desensitized receptors with naïve functional ones within or nearby the post-synaptic density. This participates to the recovery from depression in the tens of millisecond time range, in parallel with recovery from desensitization. In addition, we now show that the Ca<sup>2+</sup>/Calmodulin-dependent protein kinase II (CaMKII), which is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs) during both development and plasticity, induces the synaptic trapping of AMPARs diffusing in the membrane. Furthermore, this CaMKII dependent AMPAR immobilization regulates short term plasticity. Thus, NMDA dependent Ca<sup>2+</sup> influx in the post-synapse trigger a CaMKII and Stargazin dependent decrease in AMPAR diffusional exchange at synapses that controls synaptic function.

#### L4

##### **HIPPOCAMPAL INTERNEURON TYPES SPECIFICALLY RELATED TO COMPLEX BEHAVIOURS**

**Freund T.**

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

The morphological, neurochemical and electrophysiological characterization of inhibitory, GABAergic cell types in the hippocampus allowed the introduction of a detailed functional classification. Several lines of evidence underlies a division of labor between major interneuron classes in cortical information processing. The latest results led to the hypothesis that specificity can go as far as activity patterns of individual cell types are directly related to complex behaviours. The example used in this talk include basket cells; they control the ensemble activity and synchronization of principal neurons and are divided into two major types with different connectivities and functions: one characterized by the expression of the calcium-binding protein parvalbumin (PV) while the

other contains the neuropeptide CCK. The electrically and synaptically coupled ensembles of PV-containing basket cells are indispensable components of the oscillating cortical hardware; they represent the rigid (nonplastic) precision clockwork without which no cortical operations are possible. The activity of a similar syncytium of CCK-containing basket cells is superimposed on the PV basket cell-entrained network, conveying the emotional and motivational effects carried by subcortical pathways (containing serotonin, for example). Actions of the CCK cell ensemble are highly modifiable also by local neuromodulators and retrograde signal molecules, which may allow further fine-tuning of principal cell cooperation. Impairment of this tuning system likely results in mood disorders such as anxiety. Endocannabinoid signaling plays a crucial role in regulating postsynaptic effects of the CCK cell ensemble (i.e., the fine-tuning device) and therefore is an ideal target for the pharmacotherapy of anxiety-like behaviors.

#### L5

##### **MECHANISMS ASSOCIATED WITH LOSS OF CONSCIOUSNESS**

**Devor M.**

Department of Cell and Developmental Biology, Institute of Life Sciences, Center for Research on Pain, Hebrew University of Jerusalem, Jerusalem, Israel.

Absence of pain and loss of consciousness are the most striking characteristics of anesthesia and anesthesia-like states such as concussion, reversible coma, syncope (fainting). These states also exhibit movement inhibition, amnesia, a shift to delta-wave EEG pattern, and depressed cerebral metabolism. It is generally presumed that this constellation of signs reflects widely distributed suppression of neuronal excitability and synaptic action due to ubiquitous drug action, or oxygen or nutrient starvation. I will present evidence for a radically different architecture; that a small group of neurons in the mesopontine tegmentum has executive control over the alert status of the entire cerebrum and spinal cord, and can generate loss of pain and loss of consciousness through specific neural circuitry.

#### L6

##### **SMALL ION AND LARGE PROBLEM – CALCIUM AND ALZHEIMER'S DISEASE**

**Kuznicki J.**

International Institute of Molecular and Cell Biology, Warsaw, Poland; Nencki Institute of Experimental Biology, PAS, Warsaw, Poland.

Alzheimer disease (AD) is one of the most common forms of dementia associated with age. It affects millions of people world-

wide and there is no treatment to stop or delay its progress. The major risk factor of AD is age: statistically for 90 years old people every second will be affected. Because of a longer lifespan the number of AD patients is increasing. The disease lasts long and is devastating not only for patients, but also for the family members, who have to take 24 h care of them at later stages of the disease. All clinical trials based on the current AD hypotheses failed and some researchers predict that targeting metabolism of beta-amyloid is not the promising path to cure the disease. Thus, there is an increasing interest in searching for new potential drug targets in AD: proteins of calcium homeostasis represent some of them. Calcium signaling regulates multiple neuronal functions including synaptic transmission, plasticity and cell survival. Dysregulation of calcium homeostasis undergoes subtle changes during physiological ageing and affects neuronal function and survival. At the cellular level calcium buffering impairment, alterations in calcium entry routes into neurons, as well as mitochondrial and endoplasmic reticulum dysfunctions are observed in AD models. One of the possible targets of dysregulated calcium homeostasis in AD are proteins involved in store operated calcium entry (SOCE): Orai, calcium channel forming protein in plasma membrane and calcium sensors STIMs, located in ER. The understanding of neuronal mechanism of SOCE might help to explain the impairment of calcium homeostasis observed in AD. To identify potential drugs allowing restoring calcium homeostasis the high throughput screens are needed. We developed such screen (Honarnejad et al., abstract on AD/PD2011), which was applied to identify compounds affecting cellular calcium concentration.

## L7

### CLINICAL APPLICATION OF GENETICS IN STROKE

**Slowik A.**

Stroke Unit and Department of Neurogenetics, Jagiellonian University, Krakow, Poland.

Genetics in stroke can be considered in the following aspects: stroke in the course of inherited disorders, genetic risk factors of stroke and pharmacogenetics of stroke treatment and prevention. Although less than 1% of stroke cases are inherited, the ability to establish genetic diagnosis prevents such cases from exposure to unnecessary and potentially harmful therapeutic agents and diagnostic tests, allows introducing specific effective treatment and allows planning rational family counseling. The candidate gene approach is the most common way to study the significance of chosen genetic variants as risk factors of stroke. Unfortunately, only few genetic variants were shown to affect stroke risk in the independent replication studies. A novel approach, genome wide association studies, use the markers evenly spaced throughout genome without regard to their function or location and allows to

find all genetic variants related to the disease. Because available data suggest that the effect on stroke risk is related to many genetic variants with small effect size, large number of cases and controls are required to find such risk variants. The up-data of international effort to find out genetic variants related to stroke risk will be discussed. Increasing data indicate that several genetic factors may determine response to stroke treatment by rtPA and its prevention by aspirin, clopidogrel, warfarin, statins and anti-hypertensive drugs. The perspective for the future of stroke genetics is the era of personalized prevention and therapy, where specific biochips will help stroke clinicians to decide on the best individual prevention program and treatment.

## SPECIAL LECTURE

(Kawa.ska)

### SL

#### VISUALIZATION OF LARGE SCALE OBJECTS USING CONFOCAL MICROSCOPE LEICA TCS LSI

**Platek R.<sup>1</sup>, Korczyński J.<sup>2</sup>, Skup M.<sup>1</sup>**

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Conventional confocal microscopy is dedicated mainly to proceed with thin tissue sections and cells seeded on a cover slip. The Leica TCS LSI macro confocal is the first super zoom microscope that combines all benefits of traditional confocal microscopy with large scale imaging of anesthetized, alive objects as well as unfixed/fixed objects *post mortem*. In our experiments we have tested Leica TCS LSI for scanning of (1) murine brains and spinal cords *in vivo*, and dissected *in toto* immediately *post mortem*, and (2) fixed rat spinal cords. We used transgenic mice expressing green fluorescent protein (GFP) under PLP promoter, to visualize oligodendrocytes, and rats with spinal cords transduced with AAV vector coding for enhanced GFP under mCMV promoter, to visualize neurons and glia. Super zoom confocal microscopy let us observe general distribution of GFP-expressing cells in whole organs as well as to focus on single cells and fibers. We were able to discriminate well between main morphological features of these cells. In murine brains we could visualize myelinated axons and oligodendrocytes, whereas in rat spinal cords the extent of eGFP expressing cells and their fibers traversing along entire spinal cord could be traced. The labeled objects could be visualized from the regions lying within a range of 150 - 350  $\mu\text{m}$  from the surface of the brain/spinal cord. The details on the procedures applied, benefits and limitations of the method will be presented.

## SYMPOSIUM I

**Signaling and Plasticity  
at Local Neuronal Networks [S01]**

**S01.1**

**SLOW GABA<sub>A</sub> RECEPTOR-MEDIATED SYNAPTIC  
RESPONSE: CELL TYPES, MECHANISMS AND  
PHYSIOLOGICAL ROLE**

**Capogna M.**

Medical Research Council, Anatomical Neuropharmacology  
Unit, Oxford, UK.

GABA<sub>A</sub> receptors in the CNS mediate both fast synaptic and tonic inhibition. Over the past decade a phasic current with features intermediate between fast synaptic and tonic inhibition, termed GABA<sub>A,slow</sub>, has received increasing attention. This has coincided with an ever-growing appreciation for GABAergic cell type diversity. Compared with classical fast synaptic inhibition, GABA<sub>A,slow</sub> is slower by an order of magnitude. The speaker will summarize recent studies that have enhanced our understanding of GABA<sub>A,slow</sub>. These include the discovery of specialized interneuron types from which this current originates, the factors that could underlie its characteristically slow kinetics, its contribution to specific aspects of integrative function and network oscillations, and its potential usefulness as a novel drug target for modulating inhibitory synaptic transmission in the CNS.

**S01.2**

**SYNAPTIC CONNECTIVITY IN THE OLFACTORY  
NETWORK**

**Nusser Z.**

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Experimental Medicine, Hungarian Academy of Sciences,  
Budapest, Hungary.

The main olfactory bulb (MOB) is the first processing station of the olfactory pathway, where the axon terminals of sensory neurons establish excitatory synapses with the dendrites of mitral and tufted (M/T) cells. In the MOB the activity of the glutamatergic M/T cells is controlled by several types of GABAergic interneurons, including the granule and periglomerular cells. Our group discovered the heterogeneity of an additional interneuron type, the so called deep short-axon cells (dSACs), and revealed three distinct subpopulations. First, I will overview our results demonstrating the functional, morphological and molecular diversity of dSACs. Distinct molecular expression profiles of different dSAC subtypes will

be also demonstrated, suggesting not only different roles in the MOB circuit, but forming the foundation of future sub-type-specific genetic modifications. Later, I will summarize our recent results regarding the diversity in the cell surface distribution of GABA<sub>A</sub> receptors. By combining whole-cell recordings of mIPSCs and quantitative immunolocalization of synaptic GABA<sub>A</sub>R subunits we demonstrated that cerebellar stellate and MOB dSACs expressed only the  $\alpha 1$  as synaptic  $\alpha$  subunit, and their Zolpidem-sensitive mIPSCs had decay time constants ( $\tau_w$ ) of 4-5 ms. Nucleus reticularis thalami neurons expressed only the  $\alpha 3$  as synaptic  $\alpha$  subunit and exhibited slow, Zolpidem-insensitive mIPSCs ( $\tau_w = 28$  ms). In contrast, MOB external tufted cells contained two  $\alpha$  subunit variants ( $\alpha 1$  and  $\alpha 3$ ) in their synapses. Quantitative analysis of multiple labeled immunofluorescent images revealed small within-cell, but large between-cell variability in synaptic  $\alpha 3:\alpha 1$  ratios. This corresponded to large cell-to-cell variability in the decay ( $\tau_w = 3-30$  ms) and Zolpidem sensitivity of mIPSCs. Our results reveal a novel mechanism of generating diversity in the decay of IPSCs by independently varying the expression of different GABA<sub>A</sub>R subunits.

**S01.3**

**IMMEDIATE EARLY GENES IN PLASTICITY OF  
CORTICAL COLUMNS**

**Kossut M., Zapasnik M.**

Department of Molecular and Cellular Neurobiology, Nencki  
Institute of Experimental Biology PAS, Warsaw, Poland.

Sensory deprivation brings about rewiring of sensory cortices. Inactive inputs lose their ability to drive cortical neurons, and active inputs establish new synapses. In the vibrissae-to-barrel pathway sensory deprivation can be easily accomplished by plucking out the whiskers. Having deprived mice of all but one row of vibrissae, after 7 days we found increase in functional representation of the spared input in layer IV using [<sup>14</sup>C]-2-deoxyglucose autoradiography, Arc fluorescent *in situ* hybridization, c-Fos and Zif268 immunohistochemistry. We monitored the development of remapping in all layers of barrel columns, examining the brains after 7 and 28 days of deprivation by means of [<sup>14</sup>C]-2-deoxyglucose mapping and c-Fos immunohistochemistry. With both methods the greatest expansion of the spared input representation after longer deprivation was observed in cortical layer IV in comparison with other layers. We suggest that the characteristic strong involvement of layer IV is due to depriving this layer of a considerable part of its normal input (information coming via thalamocortical fibers) and compensatory rewiring by active inputs from other layers.

## S01.4

**ROLE OF METALLOPROTEINASES IN SYNAPTIC PLASTICITY IN THE HIPPOCAMPAL MOSSY FIBER-CA3 PATHWAY**

**Mozrzyimas J. W.<sup>1</sup>, Wójtowicz T.<sup>1</sup>, Wiera G.<sup>2</sup>, Gendosz D.<sup>1</sup>, Chmielewska M.<sup>3</sup>, Wawrzyniak M.<sup>4</sup>, Michaluk P.<sup>4</sup>, Podhorska-Okolów M.<sup>3</sup>, Dzięgiel P.<sup>3</sup>, Kaczmarek L.<sup>4</sup>**

<sup>1</sup> Laboratory of Neuroscience, Department of Biophysics, Wrocław Medical University, Wrocław, Poland; <sup>2</sup> Department of Molecular Animal Physiology, Wrocław University, Wrocław, Poland; <sup>3</sup> Department of Histology and Embryology, Wrocław Medical University, Wrocław, Poland; <sup>4</sup> Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Matrix metalloproteinases (MMPs) are widely recognized as endopeptidases involved in remodeling of extracellular matrix. Last decade studies revealed these enzymes as important regulators of synaptic plasticity and cognitive processes. In particular, MMPs inhibition led to impairment of hippocampus-dependent learning and to down regulation of long-term potentiation (LTP) maintenance in the Schaffer collateral-CA1 (SC-CA1) pathway. However, the impact of MMPs on plasticity in other hippocampal paths was not known. In our recent studies, we have addressed the impact of MMPs on plasticity in mossy fiber-CA3 (mf-CA3) projection in which, in contrast to SC-CA1, LTP expression is presynaptic. We found that pharmacological blockade of MMPs nearly abolished the late phase of LTP. Induction of LTP resulted in increased immunoreactivity for MMP-9 and enhanced gelatinase activity (in situ zymography) and these effects were associated with up regulation of de novo expression of active and latent MMP-9 forms (gel zymography). Interestingly, the late phase of LTP in the mf-CA3 pathway was reduced both in the MMP-9 KO mice and in rats overexpressing MMP-9. This finding indicates that maintenance of synaptic plasticity requires an optimal, finely tuned MMP-9 activity level. Pyramidal neurons in the CA3 region form a dense autoassociative network due to associational/commissural (AC) projections and plasticity mechanisms in AC and mf-CA3 synapses are different. Recently, we found that the late LTP phase in the AC synapses is also impaired by MMPs inhibition. Moreover, EPSC-spike potentiation in the CA3 region is strongly down regulated by MMPs blockade. In conclusion, MMPs appear to play a universal role in consolidation of synaptic plasticity in various hippocampal pathways characterized by different mechanisms of synaptic plasticity. *Supported by Ministry for Science and Higher Education grants PN/030/2006 and N N401 541540*

## SYMPOSIUM II

**Stem Cells in Neurodegenerative Diseases - Time for Clinical Translation? [S02]**

## S02.1

**THERAPEUTIC PROMISE OF ADULT STEM CELL POPULATIONS**

**Domańska-Janik K.**

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In CNS, transient global or severe focal ischemia leads to devastating irreversible cellular losses and functional impairments of the nervous tissue. Stem/progenitor cells have been considered the salvage therapy. However, besides decision on the cells source, stage of their development and proliferation, the limited survival of any implanted exogenous cells in the host tissue is one of the major obstacles for effective neurotransplantation. In preclinical animal experiment this problem gets even more complicated by necessity to use xenogeneic systems for initial testing of any human transplants and persistent lack of adequately humanized animal models. Other still unresolved questions which must be addressed are the routes, dosage and timing of cells delivery. Thus, the final answer must be at first approximated experimentally in animal models, then finally proved by case reports followed by clinical trials performed according to EBM rules. Here I will review the preclinical data gathered in our laboratory which led us toward the first, MRI monitored, clinical experiment on autologous, cord blood -derived progenitors transplantation. The neurally committed cells, prelabelled by the high signal of iron oxide nanoparticles (SPIO) were infused into the frontal horn of the lateral ventricle of 16 month old child with severe global cerebral ischemic injury. The dynamics of cell engraftment was visualized in time by MR imaging. Gradually decreased signal was noted over 4 month without any adverse side-effects. The child was followed up for next 6 month and his neurological status slightly but significantly improved. This is the first case study based on neurally -induced stem cells from the patient's frozen cord blood and considered feasible, well tolerated and safe procedure which could be monitored by MRI after intraventricular cell transplantation.

## S02.2

**CURRENT STATUS OF NON-INVASIVE CELLULAR IMAGING OF CELLS TRANSPLANTED INTO CNS: ADVANTAGES AND LIMITATIONS**

**Walczak P.**

The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Abstract not received.

### S02.3

#### HUMAN INDUCED PLURIPOTENT STEM CELLS FOR STEM CELL THERAPY IN STROKE

**Onteniente B.**

French National Institute of Health and Medical Research, Evry, France.

The potential of stem cell (SC) transplantation for the treatment of acute brain lesions has been highlighted by a number of experimental results. Among available cell sources, induced pluripotent stem cells (iPS) combine the advantages related to their similarities with embryonic stem cells (ESC), i.e., pluripotency, and self-renewal, with “à la carte” treatments. They also come with a number of potentially deleterious aspects for SC therapy that include overproliferation or abnormal differentiation due to genetic and epigenetic abnormalities. Neural progenitors (NP) were derived from 2 hiPS lines and 2 hESC lines with matching gender and were transplanted into adult Sprague-Dawley rats 7 days after a 90 min occlusion of the middle cerebral artery (MCAO). Animals were followed for behavioral tests and neurological scores and for magnetic resonance imaging (MRI) before and after MCAO, and after transplantation over 5 months. NPC from hiPSC and hESC had similar differentiation abilities *in vitro*, and displayed similar survival rate, integration and differentiation patterns after transplantation. Neurons with correct region-specific phenotype, i.e. striatal DARPP-32-positive neurons, developed with time in the grafts, in correlation with months-lasting reversal of motor deficits. Graft-derived projections were observed in several brain areas, including, but not restricted to, normal target areas of striatal projection neurons. Transplantation also significantly reduced the secondary degeneration observed in the substantia nigra pars reticulata after disruption of the striato-nigral loop, suggesting a correlation with the presence of graft-derived fibers in the area. No teratoma, overgrowth, or reversal of neuronal commitment, were observed up to 5 months. These results show that hiPSC-NPC bear similar potential than hESC-NPC for regenerative medicine in stroke and, at least partly, act by mechanisms related to integration of grafted cells into the host circuitry.

### S02.4

#### TRANSPLANTATION IN PARKINSON'S DISEASE: WILL STEM CELLS HELP TO REENTER THE CLINICAL ARENA?

**Storch A.**

Department of Neurology, Dresden University of Technology, Dresden, Germany; CRTD, Center for Regenerative Therapies Dresden, Dresden University of Technology, Dresden, Germany; DZNE, German Center for Neurodegenerative Diseases, Research Site Dresden, Dresden, Germany.

Parkinson's disease (PD) is one of the most frequent neurodegenerative disease and represents a major therapeutic challenge because of the so far missing therapeutic means to influence the ongoing loss of dopaminergic innervation to the striatum. Cell replacement has raised hope to offer the first restorative treatment option. Clinical trials have provided “proof of principle” that transplantation of dopamine-producing neurons into the striatum of PD patients can achieve symptomatic relief given that the striatum is sufficiently re-innervated. Various cell sources have been tested, including fetal ventral midbrain tissue, embryonic stem cells, fetal and adult neural stem cells and, after their groundbreaking discovery, induced pluripotent stem cells. Although embryonic and induced pluripotent stem cells have emerged as the most promising candidates to overcome most of the obstacles to clinical successful cell replacement, each cell source has its unique drawbacks. The presentation does not only provide a comprehensive overview of the different cellular candidates including their assets and drawbacks, but also of the various additional issues that need to be addressed in order to convert cellular replacement therapies from an experimental to a clinically relevant therapeutic alternative in PD. *Research of the author was supported by the Bundesministerium für Bildung und Forschung, the Deutsche Forschungsgemeinschaft (DFG) through the Sonderforschungsbereich 655 “From cells to tissues” and the DFG-Research Center and Cluster of Excellence “Center for Regenerative Therapies Dresden (CRTD)”, the Thyssen-Stiftung, and the Landesstiftung Baden-Württemberg.*

## SYMPOSIUM III

### Endovanilloids for Pain Control and Beyond [S03]

#### S03.1

#### PAIN CONTROL BETWEEN CANNABINOID AND VANILLOID RECEPTORS: COOPERATIONS AND COUNTERACTIONS

**Di Marzo V.**

Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Naples, Italy.

The transient receptor potential (TRP) channel of the vanilloid-type 1 (the “capsaicin receptor”) is involved in thermosensation, pain transduction and inflammation. It is expressed in sensory fibers of A $\delta$  and C-type, in dorsal root and trigeminal ganglia and in perivascular neurons, often together with TRP channels of the ankyrin type-1 (TRPA1, the “mustard receptor”). Whilst TRPV1 is activated by high temperatures, low pH (as during inflammatory conditions) and inflammatory mediators, TRPA1 is activated

by cold and irritants. TRPV1 activation in sensory neurons leads to release of vasodilatory peptides, thus contributing to neurogenic inflammation. TRPV1 is also expressed in central neurons of the periaqueductal grey (PAG) and rostral ventrolateral medulla (RVM), where it modulates the descending pathway of antinociception. Contrary to its role in the spinal cord and sensory afferents, TRPV1 in the PAG-RVM contributes to descending antinociception by enhancing both glutamatergic signalling/OFF neuron activity in the RVM and  $\mu$ -opioid receptor-mediated analgesia. TRPV1 is expressed in the prefrontal cortex, where it also participates in neuropathic pain. In both central and sensory neurons, TRPV1 is often co-expressed with cannabinoid CB1 receptors (the  $\Delta^9$ -tetrahydrocannabinol [THC] receptors), with which it shares two endogenous agonists, anandamide and NADA. CB1 receptor activation by these and other endocannabinoids plays a major role in the peripheral and central control of pain. TRPV1 and CB1 can either act in concert or oppose each other at modulating neurotransmitter release and pain. Furthermore, plant cannabinoids, such as THC, cannabidiol or cannabichromene, activate and subsequently desensitize TRPV1 and/or TRPA1 channels, and this property allows these compounds to influence pain and inflammation. These interactions suggest that the “endovanilloid/endocannabinoid” system is a major player in the pathophysiology of pain.

### S03.2

#### **EFFECTS OF THE ENDOGENOUS AGENT ANANDAMIDE IN ON NOCICEPTIVE PRIMARY SENSORY NEURONS**

**Nagy I.**

Department of Surgery and Cancer, Imperial College London, London, UK.

Increasing the tissue level of the endovanilloid/endocannabinoid ligand anandamide, through inhibiting its hydrolysis by the fatty acid amide hydrolase (FAAH), has been shown to produce a significant cannabinoid 1 (CB1) receptor-mediated antinociceptive effect in various animal models of pain. However, anandamide, in addition to being an endocannabinoid, is also an endovanilloid, which activates the transient receptor potential vanilloid type 1 (TRPV1) ion channel. The inhibitory CB1 receptor, the excitatory TRPV1, anandamide-synthesising enzymes and FAAH show significant co-expression in various neurons including a major sub-population of nociceptive primary sensory neurons (PSN). Hence FAAH inhibitors through modifying anandamide-mediated signalling in nociceptive PSN may compromise the CB1 receptor-mediated inhibitory effect via TRPV1-mediated excitation. Here, we will describe membrane currents induced by exogenous and endogenous anand-

amide and mediated by TRPV1 and other ion channels. Further we will also describe an intricate cross-talk between the CB1 receptor and TRPV1, which might represent a novel target drug development.

### S03.3

#### **THE ROLE OF THE DUAL FATTY ACID AMIDE HYDROLASE/TRPV1 BLOCKER, N-ARACHIDONOYL-SEROTONIN, IN INFLAMMATION AND HYPERALGESIA**

**Costa B.**

Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano-Bicocca, Italy.

Since the discovery that the cannabinoid CB<sub>1</sub> agonist anandamide (AEA) could also activate TRPV1, a barrage of investigations has focused on the relationship between these receptors, particularly in C-fibers, in which activation of TRPV1 and CB<sub>1</sub> receptor lead to nociceptive and antinociceptive effects, respectively. Consistent with the requirement for their close anatomical localization to enable a functional crosstalk, many studies reported a high level of coexpression of CB<sub>1</sub> and TRPV1 in DRG and CNS neurons. The hypothesis raised from many studies is that the crosstalk between the endocannabinoid and endovanilloid system can be pharmacologically suitable to develop drugs therapeutically effective in the field of pain and inflammation. The development of TRPV1 antagonists for acute and inflammatory pain have resulted in some unwanted side effects including increasing body temperature and some uncertainty in such antagonists for drug development. Similarly, the therapeutic employment of cannabinoid system modulators has been less simply than expected from the preclinical studies data. Although TRPV1 is a promiscuous channel, such complimentary pathways may lead to novel pharmaceutical targets. The endogenous cannabinoid system may be one such pathway that when attenuated may result in the inhibition of some types of inflammatory pain. On this basis, we tested the hypothesis that one possible strategy to retain/ameliorate the beneficial properties of cannabinoid system modulators and TRPV1 antagonists with a simultaneous decrease in the potential unwanted effects may be the employment of a molecule in which is concentrate the capability to interfere both with the cannabinoid and vanilloid system. The example showing in this presentation is the effect of the dual fatty acid amide hydrolase/TRPV1 blocker, N-arachidonoyl-serotonin, in inflammation and hyperalgesia. Consistent with the efficacy of this approach we report for the first time that the systemic administration of this dual blocker prevented the development of carrageenan-induced oedema and hyperalge-

sia. In conclusion, by combining the features of FAAH inhibitors and TRPV1 channel antagonists, we obtained a molecule that exhibits notable anti-inflammatory and anti-hyperalgesic activity in preclinical model of acute inflammation, that lacks of main side effects and that, in due turn, could be used as a pillar to evolve new drugs.

#### S03.4

##### AN IMPACT OF ENHANCED ENDOCANNABINOID/ENDOVANILLOID NEUROTRANSMISSION ON THE BEHAVIORAL EFFECTS OF COCAINE IN RATS

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The endocannabinoid system consists of cannabinoid (CB) receptors CB1 and CB2, several endogenous ligands (e.g., anandamide and 2-arachidonoylglycerol ; 2-AG), and many membrane-bound metabolizing enzymes. Endocannabinoids and mainly CB1 receptors are important for regulation of goal-maintained behaviors as well as for different pathologies affecting these processes. CB1 receptors modulate function on dopamine transmission through indirect mechanisms involving GABA or glutamate neurons while anandamide and certain eicosanoid-derived cannabinoids may also directly activate transient receptor potential vanilloid-1 (TRPV1) channels found in some dopaminergic pathways, thus allowing a direct regulation of dopamine function. Recent, pre-clinical reports indicate, that drugs affected endocannabinoid/endovanilloid system may play key role in drug addiction, especially in reinstatement of cocaine seeking behavior (Filip et al. 2006). Latest results coming from our laboratories indicate a role for CB1, CB2 or TRPV1 receptors to control food self-administration but not cocaine rewarding actions. We also report inhibitory effects of CB1, CB2 or TRPV1 receptor antagonists on drug-primed cocaine-seeking behavior. Only CB1 receptor antagonism attenuated cue-induced reinstatement of cocaine seeking. *Ex vivo* autoradiography (CB1 receptor binding measurements) and the LC/MS system (endocannabinoid/endovanilloid levels) revealed that chronic cocaine (either active or passive administration) evokes up-regulation of rat brain CB1 receptors lasting until 10 days of cocaine withdrawal while self-administered cocaine (but not given passively in a “yoked” procedure) modulates the brain levels of anandamide and 2-AG. *This study was supported by the grants by the Ministry of Science and Higher Education (Poland) to P.A. and B.B., as well as by the statutory activity of the Institute of Pharmacology Polish Academy.*

## SYMPOSIUM IV

### Neural Stem Cells and Biomaterials [S04]

#### S04.1

##### NANOTECHNOLOGY IN REGENERATIVE MEDICINE OF THE BRAIN AND SPINAL CORD

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The use of nanotechnology in cell therapy and tissue engineering offers promising future perspectives for treatment of brain and spinal cord injury. Stem cells have been shown to selectively target injured brain and spinal cord tissue and improve functional recovery. To allow cell detection, nanoparticles based on a superparamagnetic iron-oxide core or gadolinium complexes can be used to label transplanted cells. MRI is then a suitable method for the *in vivo* tracking of grafted cells in the host organism. In addition, nanoparticles based on a perovskite core can be used for tumor thermoablation. To improve MR imaging and labeling efficiency when compared to commercial contrast agents, superparamagnetic iron-oxide nanoparticles can be modified with different coatings (Poly-L-lysine, D-mannose, polydimethylacrylamide). CNS, and particularly spinal cord, injury is accompanied by tissue damage and the formation of physical and biochemical barriers that prevent axons from regenerating. One aspect of nanomedicine is the development of biologically compatible nanofiber or polymer scaffolds that mimic the structure of the extracellular matrix and can serve as a permissive bridge for axonal regeneration or as a drug-delivery system. These scaffolds, when implanted into acute or chronic spinal cord injury, provide a suitable environment not only for axonal ingrowth, but also for the growth of blood vessels and Schwann cells myelinating the axons. The incorporation of biologically active epitopes and/ or the utilization of these scaffolds as stem cell carriers may further enhance their therapeutic efficacy. *Supported by AV0Z50390703, GACR203/06/1242, IM0538, LC554 and IAA 500390902.*

#### S04.2

##### NEURAL STEM CELLS AS THERAPEUTICS AND CELLULAR MODELS OF DISEASE

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Neural stem cells have considerable potential as therapeutic agents in their own right, but also as models of the pathophysiology of brain disease. We have used two approaches to generate



human neural stem cells: conditional immortalisation, and somatic cell reprogramming (iPS cells). We have demonstrated that the conditionally-immortalised cells have efficacy in animal models of stroke and spinal cord injury. In this presentation, I outline those data, and describe the pathway that has led to the investigation of the efficacy of these cells in clinical trials in stroke. The challenge currently is to understand the mode of action of these cells, and I describe experiments that indicate an effect of engraftment on endogenous repair mechanisms. In further studies, we have attempted to enhance the tissue reconstruction capacity of these cells by combining them with matrices. I also describe the challenge to the use of these cells posed by the intrinsic diversity of neural stem cell populations, and the potential of the cells to model disease in culture.

#### S04.3

##### **MICROFABRICATION TECHNIQUES AND SURFACE FUNCTIONALISATION FOR THE CULTURE OF NEURAL STEM CELLS**

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We present different processes used for the patterning and growth of stem cells based on microspotting, microcontact printing and 3D patterning. Microspotting and microcontact-printing technique have been performed to produce micropatterned surfaces for cell-biological applications. Biomolecules have been microspotted and microstamped on plasma-polymerized poly ethylene glycol substrates. The patterns exhibited a firm stability and an improved feasibility for controlling cell localization, proliferation and even differentiation. Production and application of 3D substrate by combination of lithography and in situ UV cross linking of photoresist are also detailed. An application of these processes is the biofunctional surfaces were incubated with suspensions of human umbilical cord blood neural stem cells (HUCB-NSC). It was clearly observed how the cells adhered and grew in the protein patterned regions. After 4 weeks of culturing the cells were still anchored in the patterns. Immunocytochemistry studies indicate that cell differentiation can be controlled by the combination of interface engineering and culture conditions.

#### S04.4

##### **BIOACTIVE DOMAINS CONTROLLING NEURAL STEM CELLS FATE DECISIONS**

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Studying *in vitro* mutual interactions of the stem cells with the components of their microenvironment mimicking *in vivo* conditions is crucial for the further tissue engineering and regenerative medicine applications. Bioactive domains obtained by nano/micro emerging technologies, such as microcontact printing or piezo-electric spotting, were designed to reflect the stem cell niche composition and to influence their fate decisions. Different geometry of biofunctionalized surfaces, obtained by microcontact printing of poly-L-lysine or fibronectin, was created to study the adhesion, migration, proliferation as well as the differentiation of human cord blood-derived neural stem cells (HUCB-NSCs). The bioactive domains microspotted on the cell repellent surface contained extracellular matrix protein - fibronectin enriched with small signaling molecules, (CNTF, Jagged, Wnt, Shh, Dkk) were designed to activate particular molecular pathways leading to the maintenance of the self renewal of non-differentiated cells or to promote their differentiation into different neural lineages. Our results revealed how to control neural stem cell fate decisions by manipulating with the architecture and composition of the niche bioactive domains *in vitro*. Moreover, such domains can be used to investigate the stem cell response to cyto- or neurotoxins (MeHgCl), thus establishing good tool for screening the effect of diverse factors influencing neural stem cell development. *Sponsored by Polish Ministry of Scientific Research and Higher Education grant No: 2211/B/P01/2010/38 and European Commission Joint Research Centre NanoBioscience Action.*

## SYMPOSIUM V

### **Molecular mechanisms of neurodegenerative diseases [S05]**

#### S05.1

##### **GENETIC MODELING OF NEURODEGENERATIVE DISEASES IN MICE**

**Kreiner G.**

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Neurodegenerative diseases are characterized by profound loss of certain neuronal populations and are associated with mitochondrial and proteasomal dysfunction, alteration of cellular defense mechanisms and oxidative stress. However, the exact molecular mechanisms of neurodegeneration remain to be unraveled and current pharmacotherapy provides only symptomatic cure. Although genetic mutations responsible for familial cases are known (e.g. in Alzheimer's and Parkinson's disease), genetic animal models often do not cover all the cardinal pathological features. This is also true for transgenic models of Huntington's

disease – one of the few neurodegenerative diseases with a known genetic cause. We applied a novel approach to generate mouse models of neurodegenerative diseases based on the activation of an endogenous suicide mechanism achieved by genetic ablation of the transcription initiation factor IA (TIF-IA). Loss of TIF-IA blocks the synthesis of ribosomal RNA leading to nucleolar disruption and p53-mediated apoptosis. We used conditional inactivation of the gene encoding TIF-IA by the Cre/loxP system to induce selective loss of different neuronal populations in mice. Deletion of TIF-IA leads to rapid loss of neuronal progenitors and progressive loss of postmitotic neurons. In dopaminergic neurons and striatal dopaminergic neurons nucleolar disruption results in mutants showing respectively the typical phenotype of either Parkinson's disease (preferential degeneration of dopaminergic neurons in substantia nigra, depletion of dopamine in the striatum and typical motor dysfunctions) or Huntington's disease (loss of medium spiny neurons in striatum, impairment of motion control and clasp behavior). In addition, cellular changes associated with nucleolar disruption recapitulate some events associated with neurodegeneration in response to oxidative stress. These mutant mice may contribute to the identification and validation of new therapeutic targets.

#### S05.2

##### CELLULAR STRESS, NUCLEOLAR DAMAGE AND NEURODEGENERATION

**Parlato R.**

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The nucleolus regulates its activity in favorable or adverse conditions to optimize the cellular resources. Decreased rRNA synthesis is associated with aging and is present in age-related neurodegenerative disorders. Among the causes of neuronal death, reduced neurotrophic support and increased oxidative stress lead to down-regulation of rRNA synthesis and consequent nucleolar disruption ("nucleolar stress") making this organelle a critical sensor and mediator of the cellular stress response. Inhibition of rRNA synthesis leads to a condition of chronic stress by the stabilization of the tumor suppressor p53. p53 is a convergence point in the molecular pathways leading to different neurodegenerative diseases. However, depending on the stress signals p53 induces a variety of responses (e.g., cell-cycle arrest, senescence, apoptosis) with protective and detrimental effects. For therapeutic interventions identifying the elements that define a particular p53-mediated outcome remains a central question. To explore the impact of nucleolar stress on selective neuronal survival, we developed genetically modified mice in which the transcription factor TIF-IA, essential for rRNA synthesis, is ablated in specific neuronal

populations by the Cre-loxP system. Inhibition of rRNA synthesis and nucleolar disruption in either dopaminergic neurons or medium spiny neurons of the striatum leads to severe oxidative damage, progressive neuronal loss and typical motor dysfunctions. Gene expression profiling and biochemical assays accompanied with electron microscopy analysis, reveal the downregulation of the PI3K/mTOR signaling and activation of neuroprotective responses, such as autophagy, prior to cell death. These analyses highlight the role of the nucleolus as mediator of the stress response during neurodegeneration and provide mechanistic insights into the modes of action of p53 in the neuronal-specific responses to chronic stress.

#### S05.3

##### THE ROLE OF CREB IN NEURODEGENERATIVE DISEASES

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The cAMP response element binding protein (CREB) is an essential regulator of stimulus-driven gene expression, best described for its role in immediate-early gene transcription and neuronal plasticity. Together with the closely related cAMP response element modulator (CREM), it has been shown to be necessary for survival of specific types of forebrain neurons, most notably in the striatum or hippocampus, but dispensable in other neuron types, like the monoamine cells. This selective role of CREB in neuronal survival prompted investigation into its involvement in neurodegenerative disorders, particularly Huntington's and Niemann-Pick type C diseases. In order to assess the role of CREB-dependent transcription in triggering neuronal death, using the Cre/loxP system we have generated mice with a selective ablation of CREB in the forebrain and deletion of CREM. Gene expression profiling in the striatum and hippocampus of double-mutant mice revealed that neurodegeneration was accompanied with strong increase in abundance of transcripts associated with activation of the glia, but also changed expression of genes participating in sterol metabolism. Moreover, comparison of expression profiles with those reported in other models of striatal neurodegeneration reveals a common pattern of changes, involving several genes associated with the medium spiny neurons of the striatum. Interestingly, in a mutant mouse with a single CREM allele, no neurodegeneration was observed, and their expression profile allows to identify key CREB/CREM-dependent transcripts essential for cell survival. These observations help to understand the roles of CREB and CREM in neuronal homeostasis and their involvement in neurodegenerative disorders affecting forebrain neurons.

**S05.4****DIFFERENTIAL FUNCTION OF DOPAMINE MIDBRAIN NEURONS IN HEALTH AND DISEASE: ROLE OF ION CHANNELS****Liss B.**

Molecular Neurophysiology, Institute for General Physiology, University of Ulm, Ulm, Germany.

The dopamine midbrain system and the activity of dopamine releasing (DA) midbrain neurons is not only involved in motor control and movement disorders like Parkinson disease but also plays a crucial role in emotional and cognitive brain functions, and related disorders like schizophrenia, drug addiction, and attention-deficit-hyperactivity-disorder.

Our main research goal is to define the functional and molecular mechanisms of different types of DA midbrain neurons, which control their distinct physiological roles and their selective transitions to disease states (Liss and Roesper 2008). By combining *in vivo* retrograde tracing with *in vitro* brain slice electrophysiology and UV-laser-microdissection, as well as with quantitative DNA analysis and RT-PCR based gene expression profiling at the single cell level (Liss and Roesper 2004, Gründemann et al. 2008), we aim to define the pathophysiological signalling-pathways that control DA neuron activity as well as activation and execution of selective disease pathways of the dopamine system, in particular in Parkinson's disease (PD). The cause for the selective and progressive neurodegeneration of DA midbrain neurons in PD still remains unclear. However, genetic and environmental factors, leading to impaired DNA integrity and mitochondrial dysfunction, as well as altered ion channel activity in DA midbrain neurons, especially of ATP-sensitive K<sup>+</sup>-channels (KATP) and L-type calcium channels (LTCCs) have been identified as important factors in PD (Liss and Roesper 2010). We focus on the role of ion-channels and receptors as their cell-specific activity directly defines neuronal activity in health and disease states (Lammel et al. 2008). To address these issues, we analyze cellular function as well as mRNA expression and DNA integrity of individual DA neurons from control mice and from respective disease mouse models as well as from *post mortem* human brain tissue.

**SYMPOSIUM VI****Neuroinformatics Advances in Massive Multielectrode Electrophysiology: From Nanoneuroengineering to Brain-Machine Interfacing [S06]****S06.1****NANOMATERIALS/NEURONAL HYBRID SYSTEMS  
FIRST STEPS IN NANO-NEUROSCIENCES****Giugliano M.**

Theoretical Neurobiology and Neuroengineering, University of Antwerp, Antwerp, Belgium.

Abstract not received

**S06.2****MATHEMATICAL MODELING OF EXTRACELLULAR POTENTIALS IN THE BRAIN: RESULTS AND POSSIBILITIES****Einevoll G.T.**

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While extracellular electrical recordings have been the work horse in electrophysiology, the interpretation of such recordings is not trivial. In general, the recorded potentials stem from a weighted sum of contributions from all transmembrane currents in all active neurons in the vicinity of the electrode contacts. However, with morphologically reconstructed neurons a straightforward computational scheme can be used to calculate the extracellular potential generated by a single neuron at any point in space, and due to the linearity of the electrostatic equations, the scheme directly generalizes to extracellular potentials generated by populations of neurons. In the talk I will briefly discuss some results from our group where this scheme has been used to illuminate (A) frequency filtering and size variation of extracellular signatures of action potentials (Pettersen and Einevoll 2008), (B) the frequency spectra and spatial range of the local field potential (LFP; Linden et al. 2010), and (C) the relationship between the LFP and multi-unit activity (MUA) with the underlying neural activity in an activated columnar population of pyramidal neurons (Pettersen et al. 2008). Next, examples of developments aided by this scheme of new analysis methods for data from multielectrode recordings such as laminar population analysis (LPA; Einevoll et al. 2007), and population firing-rate model extraction (Blomquist et al. 2009), will be briefly presented. Finally, example results from a project involving generation of test data to stimulate and aid the development and testing of automated spike-sorting algorithms for tetrode data will be shown and discussed.

**S06.3****NEUROINFORMATICS AND BRAIN-COMPUTER INTERFACES: Svarog.pl AND OpenBCI.pl****Durka P.J., Michalska M., Jędrzejewski-Szmek Z., Milanowski P.**

Faculty of Physics, University of Warsaw, Warsaw, Poland.

“Neuroinformatics encompasses the tools and techniques for data acquisition, sharing, publishing, storage, analysis, visual-

ization, modeling and simulation” (question from <http://incf.org>). In this presentation we offer freely available solutions for the first six tasks in the field of biomedical time series. Svarog (Signal Viewer, Analyzer and Recorder on GPL, <http://svarog.pl>) is a multiplatform, open source software, implemented in Java, with user friendly interface and strongly modular architecture. Reading data in different formats is based on the SignalML metadescription of time series (for details see <http://signalml.org> and Durka and Ircha 2004). Advanced mathematical methods can be added to the system as plugins. OpenBCI (<http://openbci.pl>) is an open, multiplatform and multilanguage framework for brain-computer interfaces, which naturally requires online access to the data streams from the amplifier(s). Together, these two systems combine into a complete open source solution for recording EEG in freely designed and fully controllable experimental paradigms. OpenBCI can be viewed as a device driver for Svarog, or Svarog can be treated as a signal viewer for the OpenBCI system. Since 2010, these systems provide complete software platform used in the Laboratory of Biomedical Physics (<http://brain.fuw.edu.pl>) for EEG experiments, as well as teaching at the world’s first Neuroinformatics BSc studies (<http://neuroinformatyka.pl>). As for sharing the data, we propose Poland’s first neuroinformatics portal <http://eeg.pl>.

#### S06.4

##### **SOURCE RECONSTRUCTION METHODS IN ANALYSIS OF MULTIELECTRODE RECORDINGS**

**Lęski S.**

Department of Neurophysiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland.

Local field potentials (LFP), the low-frequency part of the extracellular electric potentials, reflect dynamics of the brain at the population level. Because of technological advances it is now feasible to record LFP at tens of locations simultaneously. The interpretation of these signals is complicated by the fact that the electric signals propagate in the tissue, and the signal recorded at each position may have contributions from neurons located more than a millimeter away. Therefore it is useful to estimate and analyze the current source density (CSD), the volume density of transmembrane currents which generate the observed LFP. In the past few years new methods for CSD estimation has been developed, such as the inverse CSD, based on the inversion of the forward-modeling scheme, or the kernel CSD, which employs kernel techniques used in machine learning. I will review these methods and the available software tools.

## SYMPOSIUM VII

### **Neuronal Nuclear Architecture in Health and Disease [S07]**

#### S07.1

##### **NUCLEAR ARCHITECTURE - AN EPIGENETIC MECHANISM FOR THE REGULATION OF NUCLEAR FUNCTIONS**

**Cremer M.**

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Increasing attention has been paid in the last years to the functional relevance of higher order chromatin arrangement as an epigenetic mechanism for specific gene regulation in different cell types. Recent developments of 3D nanoscopy have provided new means to study nuclear architecture at nanometer resolution, which will help to bridge the gap from the molecular level to the level of higher-order structure. The segmental organisation in metaphase chromosomes with regard to gene density is transposed into a polar organization of chromosome territories in interphase: interphase chromatin is spatially arranged in a radial pattern with the preferential localization of gene-dense chromatin in the nuclear interior and of gene-poor chromatin at the nuclear envelope. The spatial proximity of genes in the nuclear interior may facilitate the establishment of a chromatin topography, which optimally suits the structural requirements for transcription. For highly transcribed genes activation or silencing has been associated with nuclear repositioning. Yet, the influence of transcriptional activity per se has remained a matter of discussion. That an enrichment of (transcriptionally active) genes in the nuclear interior is not mandatory, was recently shown by the observation of an “inverted” chromatin pattern in rod cell nuclei of adult animals with a nocturnal life style. This remodeling into an “inverted” pattern takes place during the postmitotic terminal differentiation of rod cells. In these cells chromatin poised for transcription, as well as highly transcribed genes are located at the nuclear periphery and gene-poor chromatin (heterochromatin) in the nuclear center. This unique organization suggests a functional significance of the nuclear architecture in the retina of nocturnal animals based on physical properties of chromatin. An “inverted” chromatin arrangement is less diffractive to light and therefore provides an advantage for nocturnal life style.

#### S07.2

##### **MULTITASKING BY THE NEURONAL NUCLEOLUS: STRESS SENSING AND NEUROTROPHIC RESPONSES**

**Hetman M.**

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The ribosome is the nexus for all cellular protein translation. Critical steps of ribosomal biogenesis occur in the nucleolus, which is a nuclear subdomain that contains tandem repeats of nucleolar rRNA genes (rDNA). Ribosomal biogenesis is initiated by the RNA-Polymerase-1 (Pol1)-mediated transcription of those genes. That process is a primary site for the regulatory inputs adjusting ribosomal production to cellular needs. Although prominent nucleolar presence has been noted in neurons nearly 200 years ago, studies that directly address significance of that structure for neuronal development and/or homeostasis started to appear only recently. Our recent work has demonstrated that Pol1 serves as a sensor of neuronal DNA damage. Thus, DNA single strand breaks and/or DNA-protein adducts but not DNA double strand breaks inhibit Pol1 leading to disruption of nucleolar structure. Unlike developmentally-restricted apoptosis, such a nuclear stress response also occurs in adult neurons that are challenged with DNA damage. In developing neurons, nucleolar stress leads to activation of p53 and the p53-dependent apoptosis. Conversely, during normal development, Pol1 is major transcriptional effector for neurite outgrowth. The pro-neuritic neurotrophin BDNF increases Pol1-mediated transcription in an ERK1/2-dependent manner while Pol1 is both necessary and sufficient for the BDNF/ERK1/2-stimulated neurite outgrowth. Finally, studies of human cerebro-cortical samples from 33 Alzheimer's disease (AD) patients and 24 age-matched controls reveal AD-associated epigenetic silencing of rDNA as rDNA promoter becomes hypermethylated. Such a change in the epigenetic landscape of the AD cortical genome appears reducing ribosomal biogenesis and stabilizing rDNA.

### S07.3

#### EPIGENETIC REGULATION OF MATRIX METALLOPROTEINASE GENE EXPRESSION IN THE RAT BRAIN

Rylski M.

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Abstract not received

### S07.4

#### ARCHITECTURAL CHANGES IN THE NEURONAL NUCLEUS DURING EPILEPTOGENESIS

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Synaptic plasticity is the ability of neurons to change the strength of their synaptic connections according to the demands of the changing environment. The phenomenon underlies cognitive functions like learning and memory, and, in its aberrant form, plays important pathogenic role in brain disorders, especially in epilepsy. It is now firmly established that long-lasting synaptic plasticity involves dramatic changes in neuronal gene expression. The mechanisms of these changes are quite well understood at the level of cis- and trans-acting regulatory factors. In contrast, the potential role of higher-order nuclear architecture in genetic regulation of synaptic plasticity and epileptogenesis has not been explored. Therefore, we have examined the structure of the nuclei in the neurons of the rat hippocampus at different time points after acute seizures, using high-resolution morphological techniques and three-dimensional quantitative analysis. Our results indicate that there is prominent reorganization of the neuronal nucleus upon seizures, involving movements of highly expressed genes and chromosomal gene clusters. Such reorganization may lead to formation of molecular factories, in which transcription, splicing, and (possibly) quality control/export of pre-mRNA occur in concert.

## SYMPOSIUM VIII

### Burying Fear Memories in the Brain [S08]

#### S08.1

##### NEURAL CIRCUIT FOR FEAR RENEWAL

Maren S.

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After extinction, fear memories are not erased rather they are inhibited. This inhibition of fear is limited to the extinction context, and fear memory returns or "renews" outside of the extinction context. It has been suggested that a hippocampal – prefrontal – amygdala circuit is involved in the contextual regulation of extinguished fear memories. In this talk I will present recent experiments using anatomical disconnections and functional retrograde tracing that map the neural circuit for fear renewal in rats. These data reveal that projections from both the ventral hippocampus and prelimbic cortex to the basolateral amygdala are necessary for the renewal of fear after extinction.

#### S08.2

##### CELLULAR BASIS OF EXTINCTION OF CONDITIONED FEAR AND ITS RENEWAL

Knapska E.

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The memory of fear extinction is context-dependent: fear, suppressed in the extinction context, can renew in other contexts, invalidating the exposure therapy. Understanding the neuronal circuits underlying fear extinction is, therefore, of clinical relevance. Recent research suggests mediation of fear extinction by highly specific neuronal circuits in the amygdala, prefrontal cortex and hippocampus. However, at the cellular level, the interrelations between these brain structures remain unclear. Using c-Fos immunohistochemistry, we found strong suggestions that the context specificity of extinction is mediated by prefrontal modulation of amygdala activity and that the hippocampus has a crucial role in contextual memory retrieval. We then aimed at characterization of amygdala neurons involved in retrieval of extinguished fear memories. The use of recently generated transgenic rats carrying gene encoding fusion of PSD-95/Venus protein enabled us to study the connections of the activated neurons. The rats were injected with two anterograde axonal transport tracers either into the infralimbic (IL) and prelimbic (PRL) cortices or into the prefrontal cortex and ventral hippocampus (vHIPP). We showed that most of the cells activated in the lateral nucleus of the amygdala (La) by the extinction training receive inputs from the IL, whereas the neurons activated by the renewal of fear mainly receive signals from the PRL and vHIPP. Such differences were absent in the central nucleus of the amygdala. This suggests that extinction and renewal activate different subpopulations of neurons in the La, and that they can be distinguished by their connections to the IL, PRL and vHIPP. We also observed different involvement of the inhibitory neurons within the La following fear extinction and fear renewal. Taken together, these data suggest an appealing possibility of increasing fear extinction and preventing fear renewal by very specific manipulations of the neurons in the La.

### S08.3

#### THE FORMIN 2 PROTEIN IS REQUIRED FOR THE REMODELING OF CONSOLIDATED MEMORIES

Sananbenesi F.<sup>1</sup>, Pinhero P.<sup>2</sup>, Rebola N.<sup>2</sup>, Kerimoglu C.<sup>1</sup>, Burkhardt S.<sup>1</sup>, Rizzoli S.<sup>3</sup>, Mülle C.<sup>2</sup>, Fischer A.<sup>1</sup>

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The brain has a remarkable capacity to undergo plasticity changes that are believed to underlie the acquisition, consolidation and maintenance of memory traces. To identify the molecular and cellular substrates of cognitive function is a major aim of modern neuroscience. A critical mechanism that has been implicated with neuronal plasticity is the regulation of the actin cytoskeleton.

Thus, we study the role of FORMIN 2 (FMN2), a protein that regulates actin dynamics, in the adult brain of mice. *Fmn2*<sup>-/-</sup> mice can normally form hippocampus-dependent associative and spatial memories but display a severe failure in adapting a once acquired memory. Thus, *Fmn2*<sup>-/-</sup> mice show dramatic impairments in reversal learning paradigms. On the molecular and cellular levels this phenotype is explained by deregulated actin dynamics in the hippocampal striatum lucidum linked to a reduced number of synaptic vesicles per active zone and led to specific changes in the physiology of mossy fiber-CA3 synapses. Our data indicates that FMN2 at the mossy fiber-CA3 synapse is required for the fine-tuning of hippocampal memory traces.

### S08.4

#### NEUROBIOLOGICAL MECHANISMS OF EMOTIONAL RESPONSES IN HIGH AND LOW ANXIETY RATS

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The individual differences in the response to aversive stimuli could be important predictor for anxiety and affective disorder. Recently, in our laboratory we evaluated model of high (HR) and low anxiety rats (LR), selected according to their behaviour in the contextual fear test (i.e., the duration of a freezing response was used as a discriminating variable) to examine the neurochemical background of differences in the individual responses to conditioned aversive stimuli. Both groups had different behavioural and biochemical profiles. During test session of conditioned freezing test, LR, had higher c-Fos activity and stronger 5-HT and CRF related immunostaining in the M2 (secondary motor cortex) and higher c-Fos in the DG of hippocampus in comparison to HR. LR vocalised more in the aversive band (22 Hz) during test session, and had higher serum levels of corticosterone and higher GABA levels in BLA. HR showed also an increase in c-Fos activity and CRF related immunostaining in BLA. We found that HR rats showed a significant decrease in the conditioned fear response over the course of two extinction sessions. Upon re-testing (24 h after the conditioned fear re-training), the fear-controlled freezing behaviour of HR rats partially returned at levels below the pre-extinction value. The behaviour of the LR group remained unchanged at each stage of the experiment. The re-exposure to conditioned fear on re-test activates the prefrontal cortex and limbic areas (increased expression of c-Fos, glucocorticoid receptor, alpha-2 subunit of GABA-A receptor, gephyrin and NR2B subunit of NMDA receptor) in HR rats.

## SYMPOSIUM IX

**Neuropeptides in Cell Death and Survival: Future Therapeutic Targets [S09]****S09.1****NEUROPROTECTION AND THE CYTOSKELETON: UPDATES ON THE DRUG CANDIDATE, DAVUNETIDE**  
**Gozes I.**

Gildor Chair, Adams Super Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel & Allon Therapeutics Inc., Vancouver, BC, Canada, Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Davunetide (NAP) that is derived from activity-dependent neuroprotective protein (ADNP) protects the neuronal and glial cytoskeleton and inhibits apoptosis (Goze 2011). Recent studies showed that NAP enhances novel object recognition in the STOP heterozygous mouse - a microtubule associated protein (MAP) - deficient model of schizophrenia (Merenlender-Wagner et al. 2010, Goze 2011). In mice overexpressing alpha-synuclein - a model for idiopathic Parkinson disease - intranasal NAP improved motor function and reduced alpha-synuclein inclusions (Fleming et al. 2011). In a mouse model of brain lesion mimicking cerebral palsy (5), NAP potently protected against ibotenate-induced excitotoxic damage in the cortical plate and the white matter of P5 mice as well as against brain lesions of P0 mice (Sokolowska et al. 2011). NAP neuroprotective effects were also demonstrated in laser-induced retinal lesions in rats. Intravitreal treatment had an early short-term effect while the effect of systemic administration was delayed and prolonged (Belokopytov et al. 2011). Intranasal NAP administration in conditions of hypobaric hypoxia resulted in increased expression of Nrf2, the master regulator of antioxidant defense system coupled to improved performance in memory in rats (Sharma et al. 2011). *In vitro*, NAP diminished the characteristic increase in axonal branching that accompanies tau depletion, protecting against katanin-based loss of microtubules that is accompanied by neurodegeneration (Sudo and Bass 2011). Together, these studies suggest that davunetide (NAP) has a broad range of neuroprotective effects, and is now being tested in a Phase II/III study of PSP (Allon Therapeutics Inc.).

**S09.2****THE POTENTIAL RELEVANCE OF NEUROPEPTIDE Y (NPY) AND NPY RECEPTORS IN NEUROPROTECTION**  
**Śmiałowska M., Domin H.**

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Neuropeptide Y (NPY) is a 36-amino acid neurotransmitter and neuromodulator widely distributed in the mammalian central and peripheral nervous system and has been associated with a number of physiological and pathological conditions. In the forebrain this peptide is preferentially expressed in interneurons and modulates, mainly inhibits, the release of other neurotransmitters. NPY acts on specific receptors coupled to G-proteins, and 6 types of NPY receptors (Y1 to Y6) have been identified based on different pharmacological profiles. It has been well documented that NPY inhibits glutamatergic transmission and decreases hippocampal epileptiform activity which may lead to neuroprotection. Our earlier studies demonstrated the neuroprotective activity of NPY injected into the hippocampus on kainate neurotoxicity in that structure. The neuroprotective action of NPY against excitotoxicity was also shown in neuronal cultures. Studies with specific Y receptor ligands revealed a crucial role of Y2 and Y5 receptors. Activation of those receptors diminished neuronal excitotoxic degenerations both *in vitro* and *in vivo*, but no protective effect was observed after Y1 agonist. One of the most promising finding of our study is that NPY or Y2 and Y5 ligands exert a significant neuroprotective effect after delayed treatment, 3 - 6 h after the onset of intoxication. Much less is known about the protective action of NPY on ischemic damage, both *in vitro* and *in vivo*, but some positive effects were also found. The role of NPY in chronic neurodegenerations such as Alzheimer, Parkinson and Huntington diseases has also been postulated but the results are divergent and unclear. Summing up, our data and other authors studies indicate the neuroprotective properties of NPY; moreover, the effectiveness of delayed treatment may open up a future possibility of the potential therapeutic use of such compounds in patients.

**S09.3****OREXINS, OX RECEPTORS AND APOPTOSIS**  
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Orexins are neuropeptides synthesized by hypothalamic neurons that project throughout the brain. They regulate sleep, wakefulness, breathing, reward system and drug addiction. They strongly impact on sleep-wakefulness since orexin deficiency results in narcolepsy and cataplexy. Functions of orexins have been also described in a few peripheral tissues. The actions of orexins are mediated by two G protein-coupled receptors (GPCR) OX<sub>1</sub>R and OX<sub>2</sub>R. Classically, activation of OXRs induces cellular calcium transients through coupling to Gq proteins. An unexpected and fascinating aspect of orexins has recently emerged when we showed that orexins induce dramatic apoptosis in colon cancer

cells. I will present recent data related to the apoptotic actions of orexins and the entirely novel mechanism whereby OX<sub>1</sub>R (and probably OX<sub>2</sub>R) triggers apoptosis. Orexins induce tyrosine phosphorylation of two immunoreceptor tyrosine-based inhibitory motifs (ITIM) in OX<sub>1</sub>R. These motifs were previously considered as hallmarks of immunoreceptors. The phosphorylation of ITIMs results in recruitment and activation of tyrosine phosphatase SHP-2 and cytochrome c-mediated mitochondrial apoptosis. This mechanism is independent of Gαq-mediated activation of phospholipase C but dependent on Src stimulation by Gβγ upon OX<sub>1</sub>R activation. Finally, I will speculate on: 1) the potential importance of ITIMs in the large family of GPCRs. We show that ITIM-containing proteins represent roughly 1% of all genomes but up to 85% of human GPCRs. ITIMs are well conserved during phylogenesis and their possible role in GPCR function will be discussed; 2) the reason why adult brain neurons which express orexin receptors and are stimulated by endogenous orexins do not undergo devastating apoptosis. Since aberrant neuronal death is an outstanding feature of neurodegenerative diseases and mitochondrial death-route is important during brain maturation, the role of orexins in these processes is an open question.

#### S09.4

##### **EFFECTS OF OREXINS ON SURVIVAL OF THE PRIMARY NEURONAL AND GLIAL CELL CULTURES** Sokolowska P.<sup>1</sup>, Urbańska A.<sup>1</sup>, Biegańska K.<sup>1,2</sup>, Namiecińska M.<sup>1</sup>, Zawilska J.<sup>1,2</sup>

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Orexins (hypocretins) are hypothalamic peptides present in neurons that project throughout the brain. They act through two receptors: OX<sub>1</sub> and OX<sub>2</sub>. Recently, it has been demonstrated that orexins exert potent proapoptotic activity in various cancer cell lines. On the other hand, little is known about the role of these peptides in survival of neurons and their supportive cells in the brain. Therefore, the aim of our study was to investigate whether orexins are implicated in receptor-mediated survival- or death-promoting effects in cultured neurons and astrocytes derived from rat cerebral cortex. Real-time PCR experiments indicated that both types of orexin receptors were expressed in rat neurons and astrocytes. In cultured neurones OX<sub>2</sub>R expression was considerably higher than that of OX<sub>1</sub>R. In astrocytes similar expressions of both types of orexin receptors were identified but they were markedly lower compared to neurons. Incubation of primary neuronal cultures with orexin A, orexin B and [Ala11-D-Leu15]orexin B (a selective agonist of OX<sub>2</sub>R) resulted in a marked increase of cells viability and a parallel reduction of apoptotic cells as assessed by

MTT test and caspase-3 assay kit. In cultured astrocytes the tested neuropeptides increased cell viability (MTT) and stimulated <sup>3</sup>H-thymidine incorporation but had no effect on caspase-3 activity, an observation indicating that orexins may affect astrocytes survival by enhancing cell proliferation. In the next set of experiments cultured neurons were subjected to hypoxia induced chemically by iron chelator cobalt chloride. Orexins A and B, and [Ala<sup>11</sup>-D-Leu<sup>15</sup>]orexin B effectively protected neuronal cells, suggesting that the peptides may be endowed with neuroprotective potential in the brain. *Supported by MNiSW (grant No 4254/B/POI/2010/38) and InterMolMed (grant No POIG.01.01.02-10-107/09).*

## SYMPOSIUM X

### **Ca<sup>2+</sup> Signaling in Neuronal Development, Function and Degeneration [S10]**

#### S10.1

##### **CALCIUM SIGNALING NETWORKS IN NEURAL PROGENITOR CELLS CRITICALLY CONTROL CELL DIVISION**

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The complex and diverse cell signaling cascades underlying neural progenitor cell division and differentiation remain poorly understood. Here we demonstrate that neural progenitor cells are forming calcium (Ca<sup>2+</sup>) networks in the embryonic brain that critically control cell division. This was observed in both BrdU-pulsed animals and mouse embryonic stem (ES) cell-derived neural progenitors. The intracellular Ca<sup>2+</sup> signal cascade is driven by electrical activity and influx of Ca<sup>2+</sup>-ions from the extracellular space. Mathematical cross-correlation analysis reveals more developed networks and stronger synchronicity of spontaneous Ca<sup>2+</sup> activity in differentiated ES cells as compared to undifferentiated cells. The signaling mechanisms were independent of synaptic transmission and indicated that gap junctions were playing a major role in this signaling event. In summary, our results suggest a novel function for Ca<sup>2+</sup> signaling networks in regulating cell division of neural progenitor cells.

#### S10.2

##### **A ROLE FOR THE CA<sup>2+</sup>-DEPENDENT REPRESSOR DREAM IN THE ONSET AND PROGRESSION OF HUNTINGTON'S DISEASE**

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Deregulated intracellular  $\text{Ca}^{2+}$  homeostasis underlies synaptic dysfunction and is a common feature in neurodegenerative processes. DREAM/calsenilin/ KChIP-3 is a multifunctional  $\text{Ca}^{2+}$  binding protein with specific functions in different subcellular compartments. In the nucleus, the  $\text{Ca}^{2+}$ -free form of DREAM binds tightly to DRE sequences in the DNA and controls the expression of several genes related to  $\text{Ca}^{2+}$  homeostasis, neuronal excitability and neuronal survival. DREAM mutants unable to respond to  $\text{Ca}^{2+}$  and/or cAMP will disturb gene regulation leading to changes in the physiology of the synapses that might be determinant for or predispose to neuronal damage and death. We have used transgenic mice overexpressing dominant active DREAM mutants, i.e., insensitive to  $\text{Ca}^{2+}$ , and DREAM deficient mice to assess the role of DREAM in the onset of unbalanced motor coordination and neurodegenerative processes found in chemically- or genetically-induced mouse models of Huntington disease (HD). In addition, we have tested drugs able to bind to DREAM for an effect on the onset and progression of motor dysfunction in the R6/2 mouse model of HD. *Funded by grants from Fundacion LaCaixa, CEE (LSHM-CT-2004-512039), Era-Net-SAF2008-04753-E y CIBERNED.*

### S10.3

#### S100A6 AND ITS BINDING PARTNERS IN NERVOUS SYSTEM

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S100A6 is an EF-hand calcium binding protein belonging to the S100 family (Lesniak et al. 2009). In the  $\text{Ca}^{2+}$ -bound form S100A6 is able to interact with several ligands. More recent discoveries concerning S100A6 targets showed that it is able to form complexes with CacyBP/SIP or Sgt1. Since expression of these two S100A6 targets in brain is very high our work was focused on elucidating the role of CacyBP/SIP and Sgt1 in brain tissue. Immunohistochemistry performed on rat brain slices revealed that CacyBP/SIP is present in neurons of the cerebellum, hippocampus and cortex (Jastrzebska et al. 2000). Analysis of CacyBP/SIP mRNA expression during rat brain development showed its highest level in the cerebellum at postnatal day 21<sup>st</sup>. This might suggest the involvement of CacyBP/SIP in development of rat brain. We also examined localization of CacyBP/SIP in cultured neurons and in brain neurons of young and aged rats (Filipek et al. 2008). The results indicate that in neurons of young rats CacyBP/SIP localization is different than in aged animals. Moreover, we found that localization of CacyBP/SIP in brain neurons is similar to that observed for tau and tubulin suggesting its involvement in cytoskeletal physiology. Regarding Sgt1, we observed its immu-

nostaining in Purkinje cells of the cerebellum, in granule cells of the dentate gyrus of the hippocampus and in multiple neurons of the cortex (Spiechowicz et al. 2006). We also compared the density of Sgt1-immunopositive neurons in cortical layers of brain sections derived from healthy aged and AD-affected individuals. We found a significant decrease in Sgt1-immunopositive neurons in the temporal, angular and posterior cingulate cortex of AD brains. Such diminished immunostaining in AD cortex points to Sgt1 as a possible marker of degenerating neurons. *This work was supported by grant N N303 548439 from the Ministry of Science and Higher Education of Poland to A.F. and by statutory funds from the Nencki Institute.*

### S10.4

#### ALZHEIMER'S DISEASE AS A CALCIUMOPATHY

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Alzheimer's disease (AD) is the most common age-related neurodegenerative dementia attributed to the amyloid beta ( $\text{A}\beta$ ) deposition in the brain. Analysis of rare familial (FAD) cases with mutations in presenilin, the proteins responsible for generation of  $\text{A}\beta$  from its precursor APP, firmed the 'amyloid hypothesis' of AD etiology. However, anti-amyloid therapies failed indicating that AD pathogenesis is more complex and involves additional mechanisms. Affected brain areas of AD patients and of animal FAD models showed increased levels of intracellular  $\text{Ca}^{2+}$ , alterations in expression levels of  $\text{Ca}^{2+}$ -signaling proteins and increased activation of  $\text{Ca}^{2+}$ -dependent enzymes. Based on these data, the ' $\text{Ca}^{2+}$  hypothesis of AD' has been proposed.  $\text{Ca}^{2+}$  contributes to the development of AD by  $\text{Ca}^{2+}$ -triggered ER and mitochondrial dysfunction, and  $\text{Ca}^{2+}$ -dependent changes in gene expression. The elevated cytosolic  $\text{Ca}^{2+}$  levels affect synaptic stability and function, and can activate death signaling. Moreover, the augmented cellular  $\text{Ca}^{2+}$  levels affect  $\text{A}\beta$  generation. In turn,  $\text{A}\beta$  generation potentiate  $\text{Ca}^{2+}$  dyshomeostasis in several ways. For example,  $\text{A}\beta$  causes impairment of NMDARs signaling while the released APP intracellular domain modulates  $\text{Ca}^{2+}$  homeostasis as the regulator of IP<sub>3</sub>-mediated  $\text{Ca}^{2+}$  efflux from the ER. Mutant presenilins contribute to  $\text{Ca}^{2+}$  dyshomeostasis as impaired ER  $\text{Ca}^{2+}$  leak channels and via interactions with  $\text{Ca}^{2+}$ -signaling proteins such as calsenilin. Taken together, a growing body of evidence indicates that AD pathogenesis is based on the interplay between  $\text{Ca}^{2+}$  dyshomeostasis and neuropathological hallmarks of AD such as  $\text{A}\beta$  and mutated PS1. Thus, stabilizers of neuronal  $\text{Ca}^{2+}$  homeostasis and signaling may have therapeutic potential for AD treatment.

## SYMPOSIUM XI

## Circadian Plasticity in the Nervous System [S11]

## S11.1

## NYCTHEMERAL CHANGES IN SYNAPTIC ORGANIZATION OF THE SUPRACHIASMATIC NUCLEUS

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Circadian rhythms in mammals are synchronized to the environmental light/dark cycle through photic cues perceived by the retina and reaching the time-keeper in the suprachiasmatic nucleus of the hypothalamus (SCN). We showed in rat that the photic synchronization process is associated with rearrangements of the SCN neuroglial architecture, presumably to permit adequate intercellular phasing of the multiple SCN cellular oscillators. In the SCN retinorecipient area, neurons synthesizing vasoactive intestinal peptide (VIP), a main target for retinal signals, contribute to such reorganizations through day/night changes in the extent of their membrane coverage by glial processes and axon terminals. Using confocal imaging and electron microscopy, we further provided evidence in rat that the daily changes in axonal coverage of the VIP neurons reflected synaptic reorganizations at their surface and involved both glutamatergic terminals, known to play major roles in conveying light environmental signals to the SCN, and non-glutamatergic terminals. However, although it appeared that the whole architecture of the SCN cellular assemblage reorganized over the 24-h cycle, the density of GABAergic synapses onto the VIP neurons did not change with time of day, at least on the dendritic compartment of these neurons as assessed by electron microscopy. Taken together, these data are interpreted on the basis of accumulated evidence that the VIP neurons exert major roles in communicating temporal cues through the SCN and in synchronizing SCN oscillating neurons with each other and with the environment. Data in adrenalectomized rats indicated that the daily plastic events in SCN are regulated by the daily secretory cycles of glucocorticoid hormones, known to act as temporal endocrine signals in the modulation of photic synchronization. Additional data supporting a role for PSA-NCAM, serotonin and BDNF as molecular actors of SCN day/night structural plasticity will be also highlighted.

## S11.2

## DAILY PLASTICITY OF THE MOUSE BARREL CORTEX

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The reception of sensory information is an active process in which sensory input and motor behaviour influence each other. Mice explore the environment by rhythmically moving their whiskers. As a result, their representations in the somatosensory cortex are modulated. In the present study, we analyzed daily structural changes in the barrel cortex associated with locomotor activity level of the C57/BL mouse strain. The locomotor activity was monitored using running wheels. In a day/night regime (LD 12:12), maximum of the activity of animals was at the beginning of the night and minimum in the first half of the day. Using serial EM sections of the barrel cortex of mice, sacrificed in the period of their high or low locomotor activity, we found an increase in numerical density of synapses during the low activity period. In particular, we observed up-regulation of the density of excitatory synapses located on dendritic spines (mostly single-synapse spines). We also detected a slight increase in the density of inhibitory synapses, located on both dendritic shafts and spines, during the high activity period. It seems that in LD 12:12 conditions high locomotor activity of animals leads to the elimination of single-synapse spines. In conclusion, in LD 12:12 conditions there are cyclical, daily changes in the density of synapses and dendritic spines in mouse barrel cortex, which are associated with the daily locomotor activity pattern of the animal.

## S11.3

## CIRCADIAN CHANGES IN SYNAPSE ULTRASTRUCTURE

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The morphology of an identified motor neuron in the fly *Drosophila melanogaster* changes rhythmically every day, with smaller synaptic boutons at night when the fly is resting than during the day when the fly is active. This rhythm is largely independent of synaptic activity, is controlled from a peripheral clock and persists for at least 40 days but is no longer detectable in older

flies. We are studying the ultrastructure of these synaptic boutons in wild-type flies, as well as mutant and transgenic flies in which synaptic activity was blocked during a short time, to investigate if the rhythmic change in bouton size includes a reorganization of synapses. So far our findings have shown circadian changes in the size of synaptic vesicles and suggest circadian changes in the numbers of synapses and associated organelles as presynaptic densities ("T-bars"), endosomes, multivesicular bodies and lamellar bodies, but not in mitochondria. We propose that in this motor neuron there is a circadian rhythm of synapse assembly when the fly is at rest in the dark phase, and disassembly over the light phase, when the fly is active.

#### S11.4

### CLOCK-CONTROLLED DAILY REMODELING OF NEURONS AND SYNAPTIC CONTACTS IN THE VISUAL SYSTEM OF DROSOPHILA

**Pyza E., Górska-Andrzejak J., Damulewicz M.**

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In the first optic neuropil (lamina) of the visual system of *Drosophila melanogaster*, the first order interneurons receive photic and visual inputs from the overlying retina photoreceptors. Next they filtrate, enhance and transmit this information to the second optic neuropil (medulla). The inputs from the photoreceptors are transmitted by means of tetrad synapses, using histamine as a neurotransmitter, to 2-3 lamina interneurons, glial and amacrine cells. Among the lamina interneurons, L1 and L2 monopolar cells show circadian morphological plasticity. Their axons swell at the beginning of both the day and night and shrink at other times of the day. These changes in neurons are offset by morphological changes of glia in the lamina. The pattern of size changes of L1 and L2 axons is correlated with the pattern of *D. melanogaster* locomotor activity, which has two peaks, in the morning and in the evening. Moreover, the tetrad synapses in the lamina show similar structural oscillations, however, the rhythm in abundance of a presynaptic protein Bruchpilot (BRP) in the photoreceptor terminals is only maintained in a day/night (LD) regime but not in constant darkness (DD). It means that the density of tetrad presynaptic elements depends on light. In contrast to the presynaptic elements of tetrad synapses, their postsynaptic partners, dendrites of the L2 monopolar cells, change their structure not only in LD but also in DD. They are largest at the beginning of day. It indicates that the number of presynaptic elements of the tetrad synapses is regulated by direct exposure of light while organization of the postsynaptic sites is controlled by a circadian clock. In result circadian morphological plasticity of the

L2 dendrites is light independent and driven by a circadian input from the circadian clock located in the brain.

## SYMPOSIUM XII

### In Search of the Role of Adult Brain Neurogenesis [S12]

#### S12.1

### GENE EXPRESSION PROFILING REVEALS A NOVEL MECHANISM REGULATING ADULT NEUROGENESIS

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Mechanisms controlling the proliferative activity of hippocampal neural stem cells (NSCs) play a pivotal role to ensure life-long neurogenesis in the mammalian brain. How metabolic programs are coupled with NSC activity remains unknown. Here we show that Spot14, previously implicated in cancer and hepatic lipid metabolism, is highly enriched in a relatively quiescent hippocampal NSC population *in vitro* and *in vivo*. Spot14 lowers the availability of malonyl-CoA, which is an essential substrate of fatty acid synthase (FASN), the key enzyme of de novo lipogenesis. We show that lipid synthesis is a highly active metabolic process in dividing NSCs and that conditional deletion of FASN in adult NSCs reduces neurogenesis, indicating that levels of lipid synthesis are associated with NSC proliferation. Thus, we here identified a functional coupling between regulation of lipid metabolism and NSC proliferation, connecting cellular metabolism with NSC homeostasis in the adult brain.

#### S12.2

### PSA-NCAM PROTEINS IN HIPPOCAMPAL ADULT NEUROGENESIS

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Polysialylated form of neural cell adhesion molecule (PSA-NCAM) has been associated with differentiation, migration, maturation processes of newly born neurons in the dentate gyrus (DG) of the adult hippocampus. PSA-NCAM is also known as a marker of immature neurons and it is expressed by newly generated neurons in the granular layer of DG, in mossy fibers, granule neurons axons, in both the hilus and the CA3 subfield. A functional analysis indicates that PSA-NCAM molecule plays an important role in the hippocampus-dependent learning and memory formation. Electrophysiological

studies also show that PSA-NCAM is involved in induction of long-term potentiation (LTP) in various regions of the hippocampus. PSA-NCAM protein level is regulated by several factors, such as stress, hormones (corticosterone), neurotransmitters (serotonin, GABA), antidepressants, substance of abuse (nicotine, morphine, cocaine) or CB1 receptor agonists. Thus, alternation in the hippocampal expression of PSA-NCAM are mandatory for the structural remodeling of synaptic connections associated with long-term memory and maturation of newly generated neurons.

### **S12.3**

#### **CYCLIN D2 KNOCK-OUT MICE AS A TOOL TO INVESTIGATE THE ROLE OF ADULT NEUROGENESIS** **Filipkowski R.K.**

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The function of adult brain neurogenesis remains elusive, although it has been suggested to play a key role in learning and memory. In the previous studies, we employed cyclin D2 knock-out (cD2 KO) mice and demonstrated apparent complete deficiency in generating new neurons accompanied by minor morphological abnormalities of the brain, including smaller hippocampal formation (Kowalczyk et al. 2004). We have shown that these mice perform and learn in several behavioral tasks: context and trace fear conditioning, novel object recognition, Morris water maze, and spatial tests in IntelliCage (Jaholkowski et al. 2009). Recently, we determined that cD2 KO mice also perform surprisingly well in two-way avoidance and Barnes maze. Additionally, cD2 KO mice were subjected to hippocampus-dependent behavioral tests not requiring learning. Mutant mice showed significant impairment in several species-typical behaviors: nest construction, digging, and marble burying. Moreover, cD2 KO mice were more active in the open field and motility chamber, and showed increased explorative behavior in IntelliCage. Notably, similar deficits in species-typical behaviors and an increase in locomotor activity were previously shown in rodents with hippocampal lesions. Recently, we have also investigated alcohol consumption in cD2 KO mice and shown that they consumed significantly more of 8-16% ethanol and demonstrated a significantly higher preference for 4 – 16% ethanol (Jaholkowski et al. 2011). We conclude that either morphological abnormalities of the hippocampal formation or the impairment in the adult brain neurogenesis (or both) alter hippocampus-dependent behaviors and ethanol consumption in cD2 KO mice without influencing their learning abilities. These results show that new neurons are not necessary for learning and sug-

gest their possible role in several species-typical behaviors as well as in ethanol self-administration.

### **S12.4**

#### **INTEGRATION OF ADULT-GENERATED NEURONS INTO HIPPOCAMPAL MEMORY CIRCUITS**

**Frankland P.W.**

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Division of progenitor cells in the subgranular zone leads to the continuous addition of new neurons to the adult hippocampus, a brain region that plays a central role in memory formation. Previously we used immunohistochemical approaches to visualize the recruitment of these adult-generated neurons into circuits supporting water maze and contextual fear memories in intact animals. We showed that integration proceeds in a maturation-dependent manner, with new neurons not integrated in significant numbers until they are 4 weeks or older in age (Kee et al. 2007, Stone et al. 2010). Our new experiments address whether, once integrated, these neurons represent an essential component of a hippocampal memory trace. To address this question we developed a diphtheria toxin-based transgenic strategy which allowed us to tag adult-generated neurons and allow them to mature, and then ablate them either immediately before or after memory formation. Removal of this population of mature, adult-generated neurons had no effect on ongoing proliferation, but produced retrograde memory deficits in three different hippocampus-dependent tasks. As similar ablation one month after training produced equivalent retrograde amnesia, these results indicate that adult-generated neurons form an essential and enduring component of hippocampal memory traces.

## **SYMPOSIUM XIII**

### **Reversal of Brain Dysfunction Induced by Aging [S13]**

### **S13.1**

#### **MECHANISMS OF BEHAVIOURAL FLEXIBILITY IN MICE – ALTERATION OF COGNITIVE DEMAND INDUCED BY AGEING**

**Riedel G.**

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Traditionally, behavioural and electrophysiological recordings in animals are performed separately; however their combined use has considerably enriched our understanding of memory-related

processes. Especially single unit recordings has paved the way and highlighted performance-specific firing characteristics of neurones in behavioural context and during ageing. Less well explored is the correlation between behavioural activity and global brain activity recorded via EEG, particularly in small rodents. This was largely due to technical limitations of hardware and software and missing features such as time-stamping of events making the mapping of behaviourally relevant global activity complex and lacking precision. This presentation summarises several years of work using EEG recordings from freely moving mice equipped with multichannel wireless microchips (Neurologger – NewBehavior). Devices were validated in multiple behavioural conditions, disease models and in combination with video-observation systems. These include observation of EEG and sleep studies in home cages, in which video-monitored ambulatory activity was compared with accelerometer-based movement detection of the Neurologger. EEG is quantified for vigilance stages, sleep signatures, and stage-specific quantitative EEG power. Longitudinal recordings are presented covering the life-span of mice from 3 – 21 months and highlight the ageing profile and physiological decline. Abnormalities from these EEG signatures are confirmed in studies on genetically or pharmacologically manipulated mice using models for Alzheimer's disease and schizophrenia to validate EEG as translational biomarker of ageing and declining cognition. A final test explores EEG during behavioural exploration and after neuronal inactivation to validate global EEG changes as biomarkers reflecting behaviour. The synchronisation of video-observation with quantitative cable-free EEG recording provides a major step towards a combined psychophysiological approach desperately needed to improve basic research and translational tools in neurosciences.

### S13.2

#### PHYSICAL EXERCISE IMPROVES MEMORY ACQUISITION AND RETRIEVAL: RELATIONSHIP WITH HIPPOCAMPAL NEUROGENESIS AND NEURONAL ACTIVATION

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Degenerative processes in the aging brain impair cognitive abilities. One of the underlying problems of the aging brain is neuronal hypo-activity and hypo-perfusion of the brain. A way to counteract these problems is to increase neuronal activation and cerebral blood flow behaviorally. Physical activity (exercise) is a way to achieve this. Enhanced physical activity can improve cognitive

functioning in rodents as well as in humans. However, many aged subjects cannot perform physical exercise at the required level needed for cognitive improvements. In a series of experiments in mice we compare the beneficial effects of two forms of exercise: one active form based on running wheels and the other, more passive one based on so-called whole body vibration (WBV). We used a spatial Y-maze test for memory assessment. A 14-days running-wheel exercise protocol revealed enhanced memory acquisition and retention during learning and reversal learning in young mice (3 months of age). This exercise protocol also significantly increased the number of maturing neurons in the hippocampus, suggesting a positive relationship between the increase in neurogenesis and the positive effects on Y-maze performance. However, this exercise protocol cannot be used adequately for aged mice because of a dramatic aging-related decline in voluntary running-wheel activity. Therefore, we examined whether WBV can improve brain functioning. WBV stimulates the brain via controlled 30 Hz vibrations based on the technique used in human powerplates. Y-maze learning was significantly improved in both young (3 months of age) and aged (24 months of age) mice, but not Y-maze reversal learning. Results showed that WBV increased c-fos expression in a WBV-specific and brain region-specific manner. Taken together, these findings indicate that WBV as a form of passive exercise is suitable for improving cognitive performance in young and old subjects and may serve as a therapy to reverse brain dysfunction due to aging.

### S13.3

#### CHROMOSOMAL COPY NUMBER VARIATION IN THE NORMAL HUMAN BRAIN AND IN ALZHEIMER'S DISEASE

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Hyperploidy, i.e., neurons with a more than diploid DNA content, might be a significant source for neuronal complexity, intercellular diversity, and evolution. Genomic instability associated with hyperploidy, however, can also lead to developmental abnormalities and decreased cellular fitness. In the normal human brain, the number of hyperploidy neurons, amounts to about 10%. In Alzheimer's disease (AD), however, this number is more than doubled. Hyperploidy neurons are increased already at preclinical stages of AD and are selectively affected by cell death during progression of the disease. These findings show that neuronal hyperploidy in AD is associated with a decreased viability. Hyperploidy of neurons, thus, represents a direct molecular signature of cells prone to death in AD. This adds hyperploidy to the list of critical molecular events that are shared between neurode-

generation and malignant cell transformation. Irrespectively of whether hyperploidy results from a lack of aneuploidy clearance during brain development or an aberrant attempt of cell cycle re-entry and DNA replication in the adult, it directs our attention to a failure of neuronal differentiation as the critical pathogenetic event and potential therapeutic target in neurodegeneration.

#### S13.4

##### **DEVELOPMENT OF AN ANIMAL MODEL OF HUMAN TAUOPATHY BY INTRACELLULAR ADMINISTRATION OF TAU PROTEIN IN RAT**

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Tauopathies are a class of neurodegenerative diseases resulting from the pathological aggregation of tau protein in brain. The best known of these disorders is Alzheimer's disease, where tau protein is deposited within neurons in the form of neurofibrillary tangles, which are formed by hyperphosphorylation of this microtubule-associated protein. Good animal model that mimic this form of age-related disease is still missing. Such a model should be characterized by: over expression, hyperphosphorylation and different cellular compartmentalization of tau in neurons, breakdown of cytoskeleton and malfunctioning of neuronal transport, and impairment of cognitive processes. We propose to develop such a model by local administration of full length tau directly into CA1 area of hippocampus in rats. Using specific pore-forming agent poly-APS we delivered tau protein through the membrane into the neurons where it is metabolized and may influence cognitive processes. Additional chronic administration of okadaic acid, a specific phosphatase inhibitor, caused tau hyperphosphorylation. Because tauopathies are age-related disorders, in our experiment we used several age-groups of animals to determine the age, in which we can provoke the morphological and cognitive impairments characteristic for tauopathy. Cognitive and neurodegenerative changes were examined with behavioral test and immunohistochemical techniques. Our data indicate that use of poly-APS enables for neuronal tau incorporation at selective brain site resulting in accelerated neurofibrillary tangle-like pathology. The major advance in the development of current tauopathy model is the determination of critical age at which it is possible to trigger morphological and cognitive impairments. This model mimics several pathologies observed in progressive dementia and could be successfully used in drug discovery to support therapeutic strategies.

## SYMPOSIUM XIV

### **Social Interactions -Beyond Anxiety [S14]**

#### S14.1

##### **SOCIAL INTERACTION IN RODENTS - CONTEMPORARY APPROACH TO ANIMAL BEHAVIOR**

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Social behavior is a form of simple communication between members of the same species - both in humans and in animals. It seems to be a quite simple phenomenon, but due to its complexity and multidimensionality is difficult to quantify and analyze. In rodents and other animals social behavior constitutes a good model of the human interpersonal functioning. Various paradigms of social interaction in rats have been successfully used to study neuronal mechanisms of anxiety, aggression, domination, social defeat, stress, autism, individual differences in emotional reactivity as well as the effects of anxiogenic and anxiolytic drugs. A key advantage of this approach is a use of natural stimulus - another conspecific animal - instead of artificial objects or elaborated tasks. In addition animal reactions, that are observed as dependent variables, belongs to their natural repertoire. However, studies with multiple animals present the researcher with special challenges, both in experiment design, measurement techniques and in the analysis of data. Modern computer technology gives assistance to traditional, human observer based behavior coding as well as it does allow development of fully automatic behavior recognition systems. In this talk contemporary approach to social interaction in rodents will be presented along with major challenges and perspectives.

#### S14.2

##### **ULTRASONIC COMMUNICATION IN RODENTS: GENES, BRAIN AND BEHAVIOR**

**Wöhr M.**

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Mice and rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals. Recently, it was demonstrated that aversive 22-kHz-USVs and appetitive 50-kHz-USVs induce call-specific behavioral responses in the receiver. While 22-kHz-USVs induce freezing behavior, indicating an alarm function, 50-kHz-USVs induce social approach behavior, supporting the notion that they serve as social contact calls. The opposite behavioral responses are paralleled by

distinct patterns of brain activation. While 22-kHz-USVs induce activation in amygdala and periaqueductal gray, 50-kHz-USVs are followed by activation in the nucleus accumbens. Social approach behavior in response to 50-kHz-USVs is regulated by the endogenous opioid system. Enhanced social approach behavior was found in morphine treated rats, whereas naloxone treatment caused its reduction. Social approach in response to 50-kHz USVs further depends on social interactions during adolescence as no preference towards 50-kHz-USVs was found in rats exposed to long-term post-weaning social isolation, highlighting the importance of social experience during adolescence for affiliative behavior. Measuring USV production and behavioral responses to USVs provides therefore a unique tool to study rodent communication. This is particularly relevant for rodent models of autism as delayed language and poor communication skills are fundamental to the diagnosis of autism. Candidate genes for autism include the SHANK family of synaptic scaffolding proteins. When tested for isolation-induced USVs as pups, Shank1 null mutants emitted fewer USVs as compared to wildtype littermates; and as adults in response to female urine, the USV production by Shank1 null mutant males was characterized by an unusual time pattern and unresponsiveness to social experience. These data support the relevance of USVs for rodent models of neuropsychiatric disorders characterized by social and communication deficits.

#### **S14.3 MEASURING SOCIAL INTERACTIONS IN DRUG DISCOVERY**

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Disturbed social behaviors are symptoms in psychiatric and neurological disorders such as depression, anxiety disorders, (negative symptoms of) schizophrenia, (behavioral symptoms of) Alzheimer's disease and autism. Many rodent models have been described, allowing assessment of various social behaviors, with different values for predicting efficacy in these disorders. Nevertheless, behavioral models based on social behavior are not commonly employed in drug discovery. An overview will be presented of four paradigms based on social behavior: dominant submissive behavior in rats, a model based on natural hierarchy which may have predictive and face validity for depression and manic phase of bipolar disorder. Social-isolation induced aggression in mice, which is a model based on environmentally-induced behavior. In addition, two pharmacologically-induced social deficit models which may have predictive validity of the negative symptoms of schizophrenia: social interaction deficits induced by the NMDA antagonist PCP in rats, and deficit in huddling induced by the dopamine D3 preferring agonist PD 12,8907.

#### **S14.4 SOCIAL INTERACTION IN RODENTS – NOW AND THE FUTURE (PANEL DISCUSSION)**

**Wieczorek M.**

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Poland.

This discussion panel is aimed to facilitate information exchange and experience sharing between scientists using social interaction (SI) paradigms in their research. It would be unique occasion to gather numerous scholars who are involved in behavioral analysis of SI as well as researches that are thinking about employing such approach. During the discussion we would like to put emphasis on several important topics: (1) what are the obstacles that suppress wider use of SI paradigms in neuroscience; (2) ethological relevance of SI paradigms versus their analytical complexity; (3) communication between scientists – the necessity of detailed paradigms description, experimental conditions and clear analytical criteria; (4) automation and computer based analysis of behavior from user's experience perspective; (5) project of web-based discussion forum with idea and data sharing; (6) custom made solutions, tips and tricks.

### **SYMPOSIUM XV**

#### **Serotonin (5-HT)<sub>2C</sub> Receptor Control Over Brain Physiology and Pathophysiology: Molecular, Neurochemical and Behavioral Correlates**

#### **S15.1 5-HT<sub>2C</sub> RECEPTOR CONTROL OF MESOACCUMBENS DOPAMINE SYSTEM ACTIVITY: BEHAVIORAL, NEUROCHEMICAL AND MOLECULAR CORRELATES** **Spampinato ., Cathala A., Maitre M., Neny M. Leggio G.M., Piazza P.V., Abrous N., Revest J.M.**

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Central serotonin 2C receptors (5-HT<sub>2C</sub>R) are known to modulate the mesoaccumbens dopamine (DA) pathway by controlling DA release in the nucleus accumbens (NAc) and DA neuronal firing in the ventral tegmental area. Studies assessing the influence of 5-HT<sub>2C</sub>Rs agonists on cocaine-induced responses, have suggested that 5-HT<sub>2C</sub>Rs can also modulate mesoaccumbens DA pathway activity independently of DA release, thereby controlling NAc DA transmission. In the present study we assessed this hypothesis by studying the influence of the 5-HT<sub>2C</sub>R agonist Ro60-0175 on cocaine-induced behavioral, neurochemical and immunohistochemical responses. The intraperitoneal administration of 1 mg/

kg Ro60-0175 had no effect on the increase in NAc shell DA outflow induced by 15 mg/kg cocaine. Conversely, Ro60-0175 inhibited the hyperlocomotion induced by cocaine or by the DA-D<sub>2</sub>R agonist quimpirole (1 mg/kg), as well as cocaine-induced increase in FOS-like immunoreactivity in the NAc. These findings, considering the tight relationship between locomotor activity and NAc DA function, demonstrate that 5-HT<sub>2c</sub>R stimulation can modulate mesoaccumbens DA pathway independently of changes of NAc DA release, thereby controlling DA transmission in the NAc.

### S15.2

#### BI-DIRECTIONAL INTERACTION BETWEEN NICOTINE AND SEROTONIN (5-HT)<sub>2c</sub> RECEPTORS – BEHAVIOURAL AND MOLECULAR ASPECTS

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Recent data point to a role of serotonin (5-HT) and its receptors, mainly 5-HT<sub>2c</sub> receptor subtype, in the effects of nicotine - the key addictive component in cigarettes. Our series of studies performed on rats showed that pharmacological blockade of 5-HT<sub>2c</sub> receptors augmented the locomotor responses to acute nicotine, while activation of these receptors diminished nicotine-induced hyperactivity, the expression of behavioural sensitisation and conditioned locomotor activity as well as depression-like behaviour evoked by nicotine withdrawal. Our more recent studies demonstrated that nicotine challenge to nicotine-sensitised rats decreased [3H]mesulergine binding to 5-HT<sub>2c</sub> receptors in the prefrontal cortex, while nicotine withdrawal reduced receptor labelling in the ventral dentate gyrus and thalamic nuclei. To identify the mechanism associated with changes in radioligand binding, we analysed the pattern of 5-HT<sub>2c</sub> receptor mRNA editing (a posttranscriptional modification that may result in functionally different receptor isoforms) following repeated nicotine administration. Interestingly, our preliminary deep sequencing data showed significant decreases in 5-HT<sub>2c</sub> receptor mRNA editing in the hippocampus of nicotine-withdrawn animals. Such an alteration in editing may affect the availability of binding sites for 5-HT<sub>2c</sub> receptor radioligand and could partially explain changes in radioligand binding noted in this brain region. Taken together, our data support the existence of bi-directional interaction between 5-HT<sub>2c</sub> receptors and nicotine. Clear effects of 5-HT<sub>2c</sub> receptor agonists to ameliorate symptoms associated with nicotine dependence have been shown. On the other hand, the ability of nicotine to affect 5-HT<sub>2c</sub> receptor binding and editing has also been reported. Present data show a new direction in the search for efficient anti-nicotinic drugs and the possibility of using 5-HT<sub>2c</sub> receptor agonists as adjuncts to smoking cessation therapy.

### S15.3

#### THERAPEUTIC POTENTIAL OF 5-HT<sub>2c</sub> RECEPTOR LIGANDS TOWARDS COCAINE ADDICTION

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Serotonin (5-HT) neurotransmission controls the brain physiology and contributes to the etiology of many neuropsychiatric disorders. One of the key modulators of 5-HT system is the 5-HT<sub>2c</sub> receptor which regulates feeding, satiety, mood and cognition as well as underlines the mechanisms of depression, schizophrenia and addiction (Filip and Bader 2009). Among abused drugs, cocaine addiction creates serious health and legal implications in developed world while no medication is approved for the treatment of cocaine addiction. Detailed preclinical pharmacological analyses with several selective 5-HT<sub>2c</sub> receptor ligands have provided consistent proofs that these receptors contribute to cocaine acute and repeated behaviors. In general, systemic pretreatment of 5-HT<sub>2c</sub> receptor agonists attenuates, while antagonists enhance cocaine-induced psychomotor activation, reward and reinforcement as well as subjective (discriminative stimulus) effects in laboratory animals (Filip et al. 2010). 5-HT<sub>2c</sub> receptors are also important neural mediators in the circuitry underlying cocaine-seeking and -taking behaviors since their stimulation attenuated conditioned hyperactivity to cocaine and the priming effect of acute cocaine, cue- or stress-controlled cocaine-seeking. More importantly, the inhibitory action of 5-HT<sub>2c</sub> receptor agonists on the reinstatement of cocaine seeking, when extrapolated to abstinent human addicts, suggest therapeutic potential for these drugs as pro-abstinence and anti-relapse ones. The main shortcoming of 5-HT<sub>2c</sub> receptor agonists for cocaine addiction may be their inhibitory effects in motivated behaviors (including food consumption) as found in preclinical research (Neisewander and Acosta 2007) and recent clinical trials (Smith et al. 2009). *This study was supported by the statutory activity of the Institute of Pharmacology Polish Academy of Sciences (Krakow).*

### S15.4

#### 5-HT<sub>2c</sub> RECEPTOR LIGANDS IN THE TREATMENT OF OBESITY AND TYPE 2 DIABETES

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The central serotonin (5-hydroxytryptamine, 5-HT) system is an established modulator of energy balance. Therefore, it is unsur-



prising that recent (e.g. sibutramine) and drug discovery (e.g. lorcaserin) obesity treatments target serotonin pathways to affect food intake, body weight, and glucose homeostasis. Pharmacological and genetic research implicates the Gq-coupled serotonin 2C receptor (5-HT<sub>2c</sub>R) specifically in these effects. We sought to clarify how serotonin in general, and the 5-HT<sub>2c</sub>Rs in particular, modulates these effects. We found that 5-HT<sub>2c</sub>R agonists require melanocortin pathways to exert effects on appetite and glucose homeostasis. Specifically, we observed that 5-HT<sub>2c</sub>Rs are co-expressed with neurons containing the endogenous anorectic melanocortin agonist pro-opiomelanocortin (POMC)/ $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) in the arcuate nucleus of the hypothalamus. We found that anorectic concentrations of 5-HT<sub>2c</sub>R agonists activated POMC/ $\alpha$ -MSH neurons. Furthermore, we observed that 5-HT<sub>2c</sub>R agonists improved glu-

cose and insulin tolerance at concentrations insufficient to influence appetite. These improvements were associated with enhanced insulin signalling in liver and muscle and reduced hepatic gluconeogenesis. In the brain,  $\alpha$ -MSH acts at the melanocortin 3 (MC3) and melanocortin 4 (MC4) receptors. To further clarify the pathway through which serotonin influences appetite and glucose homeostasis, we examined whether pharmacological blockade or genetic inactivation of the MC3Rs or MC4Rs abolishes 5-HT<sub>2c</sub>R agonist hypophagia and improvements in glucose homeostasis. We observed that activation of the MC4Rs, but not the MC3Rs, is required for 5-HT<sub>2c</sub>R agonists to influence feeding and insulin action. A model is presented in which activation of the melanocortin system is downstream of serotonin and is necessary to produce the complete effect of 5-HT<sub>2c</sub>R agonists on energy balance.